

# Combining dialysis and dilution techniques to estimate gross growth rate of phytoplankton and grazing by micro- and mesozooplankton *in situ*

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With 7 figures and 1 table

**Abstract:** Measurements of *in situ* phytoplankton growth and grazing by zooplankton normally involve different techniques. We show that a single dilution experiment based on dialysis bags can be used to do these estimates *in situ*. Dialysis bags allow an estimate of the *in situ* phytoplankton gross growth rate whereas the dilution gradient allows a simultaneous estimate of microzooplankton grazing. The comparison of the phytoplankton net growth rate outside dialysis bags and the estimated apparent growth rate of phytoplankton in undiluted samples within dialysis bags allows estimating additional loss processes such as mesozooplankton grazing. The method is especially useful in mesocosms experiments.

**Key words:** dilution experiments, dialysis bags, gross growth rate, microzooplankton grazing, mesozooplankton grazing.

## Introduction

Phytoplankton dynamics is controlled by the balance between growth and mortality. Phytoplankton production is considered to be controlled by the rate of nutrient supply or light, and final abundances and net growth rates are considered to be determined by predation pressure, by nutrient supply or by both.

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This simple framework has been routinely used to explain pelagic food web dynamics in experiments and empirical analysis of databases of phytoplankton. To study such mechanisms of pelagic food web dynamics in detail, mesocosm experiments have become an increasingly important tool in plankton research. Additionally, the understanding of those mechanisms structuring pelagic food webs demands for estimates of rates and fluxes within the food web beside biomass estimates of the different food web compartments.

The question of how close the growth rates of the phytoplankton are to maximal phytoplankton growth rates in a certain environment is important to estimate to what extent bottom-up vs. top-down factors are acting on a phytoplankton population. Several techniques can be used to study factors influencing *in situ* growth dynamics of phytoplankton. Most methods involve incubation of samples in closed bottles. The use of bottle incubations has, however, several drawbacks. The primary disadvantage lies in the chemical isolation of the incubation bottles from the surrounding water. Depending on the experimental treatment (grazer removal, dilution) nutrient ratios, supply rate and demand might strongly differ between incubation bottles and *in situ* conditions and between bottles with different treatments (FURNAS 1982). Nutrient addition at concentrations saturating gross phytoplankton growth rates has been used as a way out of the dilemma. The addition of nutrients can guarantee identical gross growth rates in bottles but the resulting gross growth rates could be different from *in situ* conditions, if nutrients are limiting.

The incubation of natural phytoplankton communities enclosed within dialysis bags suspended *in situ* is one of the most reliable approaches to estimate the *in situ* growth rates of marine phytoplankton (FURNAS 1990). This technique has been successfully used to estimate *in situ* growth rates of phytoplankton (e. g., SAKSHAUG 1977, SAKSHAUG & JENSEN 1978, MURA & AGUSTI 1996). The advantage of dialysis bag experiments is that they allow the maintenance of chemical exchange between the enclosed population and the surrounding medium, and also that they allow an estimation of growth rates for a wide range of taxa (FURNAS 1990). The major disadvantages of the estimation of algal growth rates using incubation in dialysis bags are the relatively long time required relative to other techniques (e. g. tracer incorporation) and the possibility that grazers are included. This is especially important for microzooplankton (largely nanoflagellates, ciliates and very small immature stages of metazoan grazers) which cannot be separated from phytoplankton by screening. Microzooplankton grazing can be an important source of algal mortality so that inclusion of micrograzers would strongly influence the estimation of growth rates. Moreover, grazer activity can contribute nutrients that may enhance algal growth on time – scales of days to weeks.

Three approaches have been used to study the effect of micro-grazers on prey: a) following the population dynamics of both groups during a grazing

period, b) tracing labeled prey in a water sample and c) experimental reduction of grazing pressure by dilution and measuring the growth of the prey at the different dilutions. Advantages and drawbacks of these methods have been described in detail by several authors (e. g LANDRY 1994, SHERR & SHERR 1994, VAQUE et al. 1994). Whereas the first approach has been extensively explored to follow predator – prey dynamics and the second approach has been most often employed as a tool to quantify bacteriivory, the third approach proved to be fruitful when the grazing pressure on phototrophic organism is studied.

Established methods to estimate microzooplankton grazing impact on natural communities of marine phytoplankton are dilution experiments (LANDRY & HASSETT 1982). Dilution reduces encounter rates between phytoplankton and microzooplankton. Natural assemblages of phytoplankton and grazers are diluted with filtered seawater in a dilution series. The microzooplankton grazing rate is estimated as the slope of a regression of apparent phytoplankton growth rate in the various dilutions against dilution factor. The approach relies on three assumptions (LANDRY & HASSETT 1982):

1. Individual phytoplankton growth is not directly affected by the presence or absence of phytoplankton *per se*.
2. The probability of a phytoplankton cell being consumed is a direct function of the rate of encounter of consumers with prey cells. The model assumes that the specific grazing rate does not change implying that predators are not food saturated and predators do not increase their feeding activity at low food concentrations.
3. Phytoplankton growth is exponential.

Dilution experiments are now a standard protocol for the estimation of microzooplankton herbivory (BURKILL et al. 1993, LANDRY 1993). However, incubations of the dilution series are normally done in bottles and have therefore the above described disadvantages (for a detailed discussion of dilution techniques and nutrient dynamics see ANDERSEN et al. 1991).

Here, we describe how the combination of the dilution technique to estimate microzooplankton grazing with the incubation of the dilution treatments in dialysis bags *in situ* can be used to estimate gross growth rates of phytoplankton together with microzooplankton and mesozooplankton grazing rates in mesocosms experiments. Dialysis bags have already been used to incubate dilution samples *in situ* by LANDRY & HASSETT (1982) to test whether the amount of nutrients added to natural phytoplankton communities enclosed in bottles aboard a research vessel yielded similar growth responses as *in situ* incubations. However, the combination of dialysis and dilution techniques has to our knowledge never been used regularly to incubate dilution series *in situ*. With the increasing use of mesocosm techniques in plankton research, dilution experiments in dialysis bags could become a useful tool to investigate growth and grazing dynamics of phytoplankton communities. We demonstrate in the

following the use of the method in two marine pelagic mesocosms experiments.

## Material and methods

### Marine mesocosm experiments

We carried out several series of marine dilution experiments during two large mesocosm experiments in the bay of Hopavagn, central Norway. For the first experiment we moored 10 mesocosms made from transparent polyethylene tubes to floating stands. The volume of each bag was approximately  $5\text{ m}^3$ , with a diameter of 0.9 m and a total depth of 6.5 m, consisting of a 6 m straight tube and a sealed, conical bottom. We filled the mesocosms on the evening before the start of the experiments by lifting them from 7 m depth to the surface and enclosing the natural phytoplankton and zooplankton community. Zooplankton consisted mainly of calanoid copepods of the species *Temora longicornis*, *Centropages* sp., *Pseudocalanus elongates* and *Acartia longiremis*. More than half of the biomass was copepods of the genera *Temora* and *Pseudocalanus*, which were fairly equal in biomass. The water columns in the bags did not stratify and were well mixed by wave action. We added nutrients to the mesocosms on the evening of day 1 and each of the evenings thereafter. The nutrient addition was comparable to the natural load of the system (VADSTEIN et al. 2004). Nutrients were added in an atomic ratio of 16 : 16 : 1 for Si : N : P. Si was added as silicate, P as phosphate and N as nitrate and ammonia (1 : 1). The daily doses of added P was  $0.5\ \mu\text{g P l}^{-1}\text{ d}^{-1}$ . We created gradients of predation pressure on copepods by adding different numbers of ctenophores (0, 5, 10, 20, 40 *Bolinopsis infundibulum*) to different mesocosms. All treatments were in duplicate. Ctenophores were carefully collected by net hauls with plastic bags mounted on the end of the net. Before the ctenophores were added we emptied the mesocosms from ctenophores by using a net with 1 cm mesh width and a diameter of 0.9 m. The treatments with 10 ctenophores per enclosure resembled the natural density of *Bolinopsis* in the bay of Hopavagn at the start of our experiment. Ctenophore numbers in the bags were adjusted two times per week to keep the initial gradient during the experiment. After 4 weeks, we exposed dilution experiments in dialysis bags within the 10 mesocosms (1.5 m water depth) to investigate whether our experimental manipulation of the ctenophore top predator had a cascading influence on micro- and mesozooplankton grazing rates and phytoplankton gross growth rates (see a detailed description below). Mesocosm walls were exposed to wave action and the dialysis bags were therefore incubated in a well mixed water column.

The second experiment was established to investigate the influence of silicate on trophic cascades within the same marine system. 12 mesocosms such as described above were moored in the bay of Hopavagn and fertilized with different amounts of silicate. More details about the second mesocosm experiment are described elsewhere (SOMMER et al. 2005). We performed dilution experiments in dialysis bags to estimate the effect of our experimental manipulation on phytoplankton gross growth rates, and micro- and mesozooplankton grazing three weeks after the start of the experiments. We enclosed natural phytoplankton from the mesocosms within dialysis bags along a dilu-

tion gradient and suspended the bags *in situ* in 1.5 m water depth (see a detailed description below).

## General methods for both experiments

### Dilution experiments

Bags with a volume of 250 ml were built with dialysis membrane tubes with a molecular weight cut-off of 6000. This allowed diffusion of molecules smaller than proteins which equilibrate rapidly with ambient water (<4 h, MURA et al. 1996; <8 h, STRIEBEL, unpubl. results). Dialysis tubes were hydrated by soaking them in deionized water for 12 h prior to use.

Dialysis cultures consisted of depth integrated samples from well mixed enclosures. Samples were taken with a tube sampler and filtered through a 200  $\mu\text{m}$  mesh to exclude macrozooplankton. The original sample was diluted with GF/F filtered water from the same water body in 5 steps. The share unfiltered water was 12.5 %, 25 %, 50 %, 75 % and 87.5 %. One or two replicate dialysis bags were prepared for each dilution step. Samples were incubated for 48 hours and this incubation period resulted in a clear and measurable growth response of phytoplankton in all experiments.

Changes in the phytoplankton abundance in the mesocosms in samples taken at the beginning and the end of the incubation period were assumed to represent the net rates of population change (TOTH 1980, FURNAS 1990, MURA et al. 1996).

After incubation, dialysis tubes were opened and sub samples (50 ml) were filtered onto GF/F filters and, following methanol extraction, analyzed for chlorophyll-*a* (a common tracer for dilution experiments, BÖTTJER & MORALES 2005) using a Turner design fluorometer (STRICKLAND & PARSONS 1972). Additionally 100 ml sub-samples were fixed with Lugols iodine and counted according to Utermöhl's inverted microscope technique (UTERMÖHL 1958). If possible, 400 individuals per category were counted which gives 95 % confidence limits of  $\pm 10\%$ , if cells are randomly distributed (LUND et al. 1958). Thereby we want to demonstrate the ability of the technique to follow growth and grazing of single algal species and groups. More examples about the use of this technique to follow growth and grazing dynamics of single algal species and groups can be found in SOMMER et al. 2005.

Net growth rates ( $r$ ; in  $\text{d}^{-1}$ ) in the mesocosms, epilimnion and in the dialysis bags were calculated as:

$$r = (\ln N_2 - \ln N_1)/(t_2 - t_1) \quad (1)$$

where  $N_1$  and  $N_2$  correspond to the initial and final phytoplankton concentrations,  $t_1$  and  $t_2$  to the initial and final incubation times, respectively. As a measure of total phytoplankton concentration we used chlorophyll-*a* concentrations (in  $\mu\text{g l}^{-1}$ ). In order to determine net growth rates of individual algal, species algae biovolume ( $\mu\text{m}^3 \text{l}^{-1}$ ) was used.

Grazing rates by microzooplankton ( $\gamma_{\text{micro}}$ ) were calculated by linear regression ( $y = a + bx$ ) of  $r$  in the dialysis bags on the share of unfiltered water ( $x$ ) where  $y = r$ ,  $a$  (intercept) =  $\mu$  and  $b$  (slope) =  $-\gamma_{\text{micro}}$  giving Eq. 2:

$$r = \mu - \gamma_{\text{micro}} \cdot x \quad (2)$$

$\mu$  reflects the gross growth rate under the *in situ* light and nutrient conditions, without grazers present.

Eq. 2 can be transformed to give Eq.3:

$$\gamma_{\text{micro}} = (r - \mu)/x. \quad (3)$$

Grazing rates by mesozooplankton (copepods;  $\gamma_{\text{cop}}$ ) were calculated as the difference between net phytoplankton growth rates calculated from Eq. 2 for  $x = 1$  ( $r_1$ ) and  $r$  in the mesocosms ( $r_{\text{meso}}$ )

$$\gamma_{\text{cop}} = r_1 - r_{\text{meso}}. \quad (4)$$

Here we assume that the light at the depth of incubation of the dialysis bags approximately equals the average light intensity of the mixed layer in the mesocosms. Furthermore, we also assume that, in the mesocosms, losses through sedimentation were negligible or that other losses were not different between the mesocosms and the dialysis bags. The calculated  $\gamma_{\text{cop}}$  for control mesocosms without copepods can serve as a check for the realism of these assumptions (see SOMMER et al. 2005 for a test of this assumption).

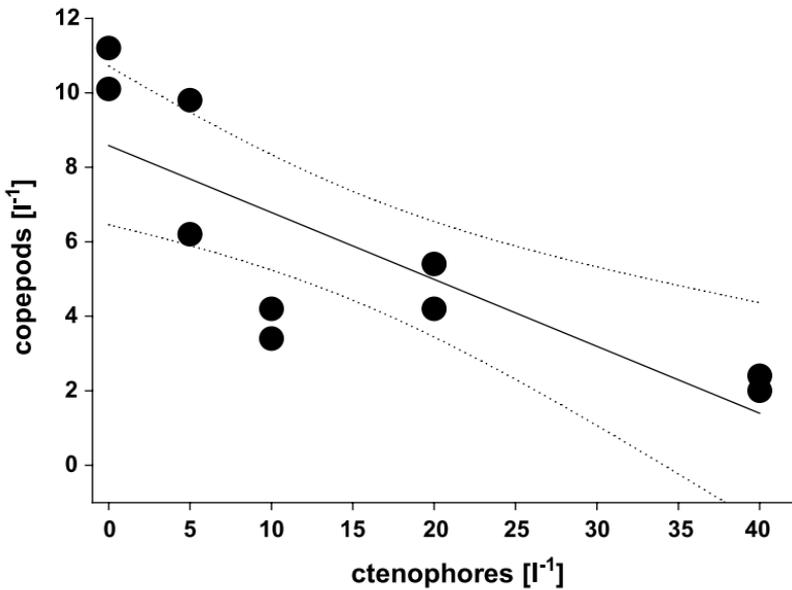
## Zooplankton sampling

Mesozooplankton samples were collected with a 200  $\mu\text{m}$  net and counted with a Leitz M3 dissecting microscope. Samples for determination of ciliate biomass were taken with a 2 m long Ramberg tube sampler. Integrated composite water samples were taken to represent the whole mesocosms from 0 to 6 m depth. Samples were settled in 50 ml Utermöhl chambers and counted in an inverted microscope. Normally >200 cells were counted per sample, which should give a coefficient of variation of <7%.

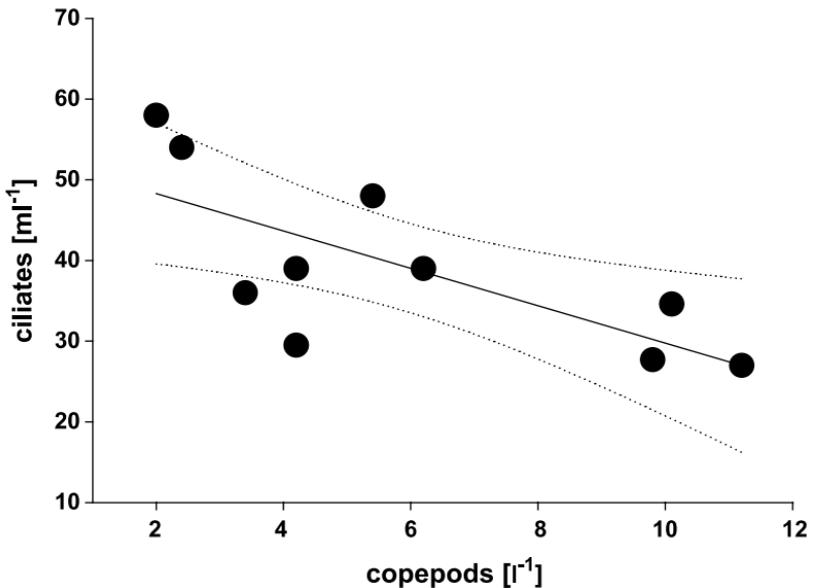
# Results

## Experiment 1

Our manipulation of the ctenophore abundances in the different enclosures influenced a trophic cascade down to phytoplankton (GELZLEICHTER 2002). Increasing ctenophore numbers resulted in decreasing copepod abundances (Fig. 1). Decreasing copepod abundances resulted in increasing ciliate abundances (Fig. 2). Copepod numbers at the time of the dilution experiments (after four weeks) were between 2 and 11 copepods  $\text{l}^{-1}$  and ciliate abundances were between 27 and 58 ciliates  $\text{ml}^{-1}$  (Table 1). These abundances are within the natural range in the bay of Hopavagn (VADSTEIN et al. 2004). Copepods showed a negative correlation with large phytoplankton (diatoms) whereas ciliates had a negative impact on small phytoplankton (GELZLEICHTER 2002). In the different dilution series apparent growth rates of phytoplankton were highest in dilute waters and decreased with the proportion of unfiltered water (Fig. 3). The estimated gross growth rates of phytoplankton (intercept,  $\mu$ ) were



**Fig. 1.** Copepod abundance as a function of initial ctenophore abundance in the mesocosms. Linear regression analysis gave:  $y = 8.63 - 0.18x$ ;  $r^2 = 0.62$ ;  $p < 0.01$ . Dotted lines represent 95% confidence intervals.



**Fig. 2.** Ciliate abundance as a function of copepod abundance in the mesocosms. Linear regression analysis gave:  $y = 52.9 - 2.32x$ ;  $r^2 = 0.51$ ;  $p < 0.05$ . Dotted lines represent 95% confidence intervals.

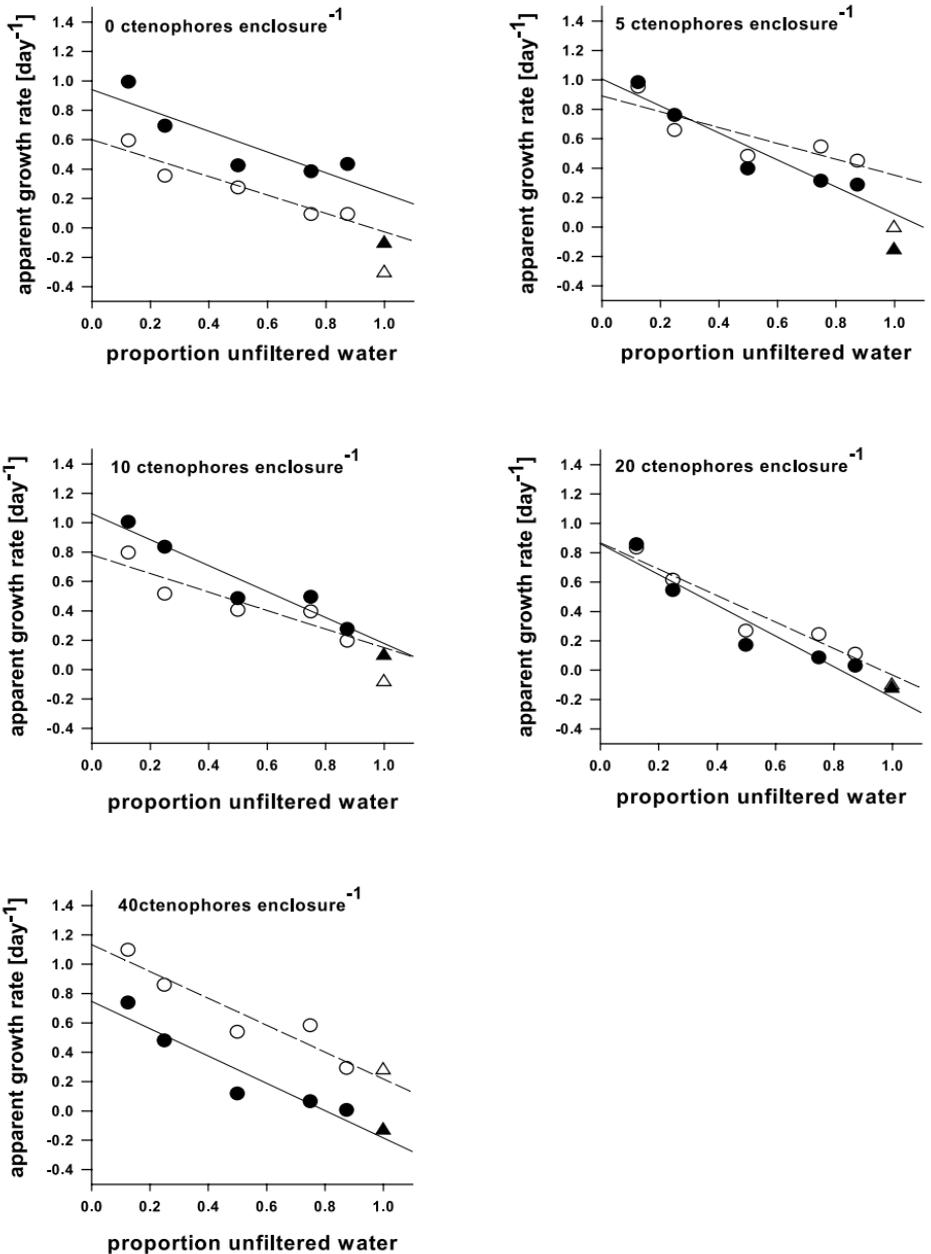
**Table 1.** Copepod abundance, ciliate abundance, phytoplankton gross growth rates and microzooplankton and mesozooplankton grazing in the different ctenophore treatments after 4 weeks of the experiment. Gross growth rates and microzooplankton grazing were estimated from linear regressions of apparent phytoplankton growth rates on dilution (Fig. 3), standard errors of these estimates are given in parentheses. A and B indicate the two replicate mesocosms per ctenophore treatment.

Treatment [ctenophores enclosure <sup>-1</sup> ]	Copepods [l <sup>-1</sup> ]	Ciliates [ml <sup>-1</sup> ]	Phytoplankton gross growth rate [day <sup>-1</sup> ]	Mikrozooplankton grazing [day <sup>-1</sup> ]	Mesozooplankton grazing [day <sup>-1</sup> ]
0A	10.1	34.6	1.01 (0.10)	0.91 (0.18)	0.36
0B	11.2	27.0	0.89 (0.12)	0.53 (0.20)	0.25
5A	9.8	27.7	0.59 (0.06)	0.62 (0.11)	0.34
5B	6.2	39.0	0.94 (0.13)	0.70 (0.23)	0.28
10A	4.2	29.5	0.78 (0.09)	0.63 (0.16)	0.05
10B	3.4	36.0	1.06 (0.09)	0.88 (0.15)	0.26
20A	4.2	39.0	0.86 (0.08)	0.89 (0.16)	-0.06
20B	5.4	48.0	0.86 (0.12)	1.04 (0.21)	0.07
40A	2.4	54.0	1.13 (0.11)	0.92 (0.19)	-0.05
40B	2	58.0	0.75 (0.10)	0.93 (0.18)	-0.06

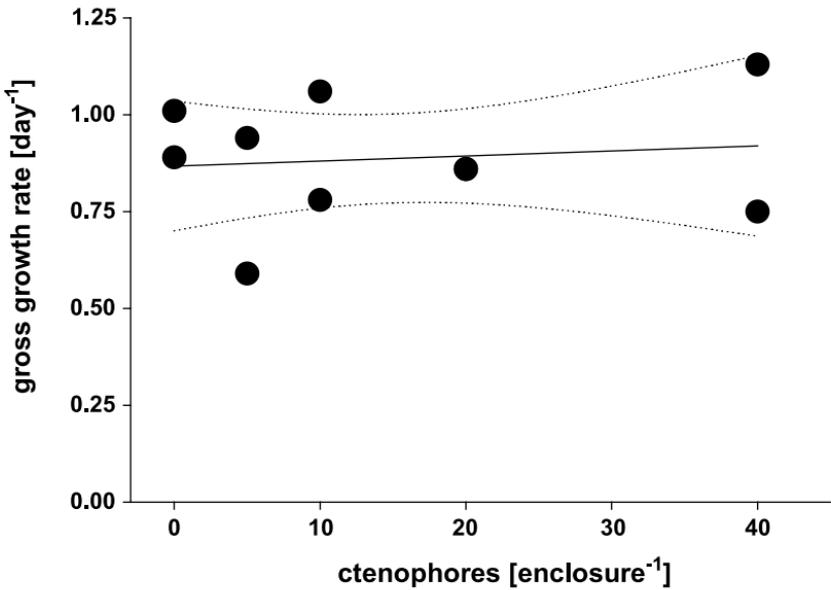
similar between treatments (Table 1, Fig. 4). However, the slopes of the regression of apparent phytoplankton growth rate to dilution (i. e.  $\gamma_{\text{micro}}$ ) differed and showed a significant linear relation to ciliate abundance (Table 1, Fig. 5). Microzooplankton grazing was between 0.65 day<sup>-1</sup> at low ciliate abundances and 0.95 day<sup>-1</sup> at the highest ciliate abundances. Enclosures with small numbers of ctenophores showed larger stocks of mesozooplankton (Fig. 1) which in turn suppressed micrograzers like ciliates (Fig. 2). Additionally, larger stocks of mesozooplankton resulted in larger mesozooplankton grazing rates ( $\gamma_{\text{cop}}$ ) on phytoplankton (Fig. 6). Our mesozooplankton grazing estimates ranged from 0.3 day<sup>-1</sup> at high copepod abundances (10–12 l<sup>-1</sup>) to around 0 day<sup>-1</sup> at densities below 2 copepods l<sup>-1</sup>. The results of our dilution experiments in dialysis bags fitted well to the general results of this and similar mesocosm experiments (GELZLEICHTER 2002, HANTZSCHE 2002, STIBOR et al. 2004, VADSTEIN et al. 2004).

## Experiment 2

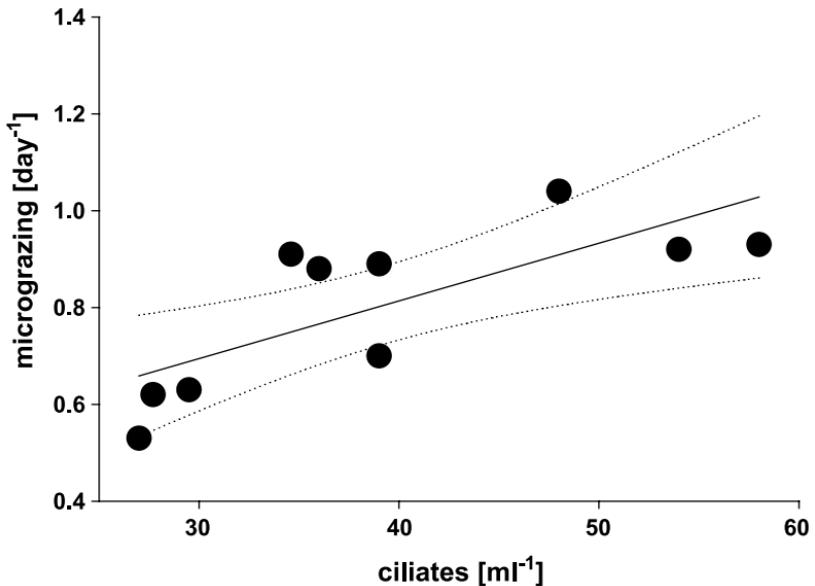
Fig. 7 shows two examples of phytoplankton gross growth rates and micro- and mesozooplankton grazing rates from the second mesocosm experiment. The figures are based on cell count data, demonstrating the use of the method to estimate growth parameters of individual algal groups. Both examples are taken from the same mesocosm, which had been stocked with a natural density of copepods (approximately 20 l<sup>-1</sup>). The apparent growth rate of nanoflagella-



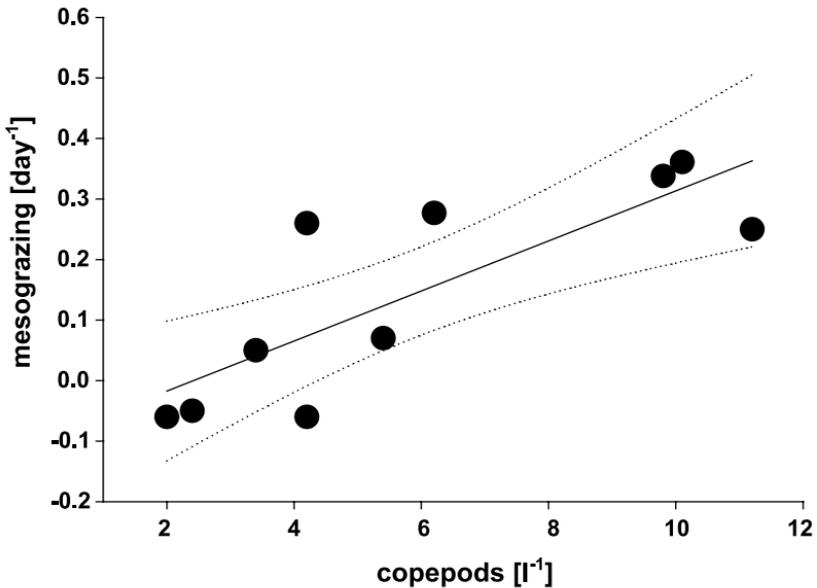
**Fig. 3.** Apparent growth rate of phytoplankton (circles) in dilution series in mesocosms with different ctenophore densities (0–40 individuals per mesocosms). Triangles indicate apparent growth rates in mesocosms ( $r_{\text{meso}}$ ). The difference between the regression line and  $r_{\text{meso}}$  is an estimate of mesozooplankton (copepod) grazing ( $\gamma_{\text{cop}}$ ). Open and filled symbols represent the two replicate mesocosms per ctenophore treatment.



**Fig. 4.** Gross growth rate of phytoplankton in the different ctenophore treatments. Linear regression analysis gave:  $y = 0.87 + 0 x$ ;  $r^2 = 0.01$ ;  $p = 0.73$ . Dotted lines represent 95 % confidence intervals.



**Fig. 5.** Microzooplankton grazing as a function of ciliate abundance. Linear regression gave:  $y = 0.34 + 0.01 x$ ;  $r^2 = 0.58$ ;  $p = 0.01$ . Dotted lines represent 95 % confidence intervals.

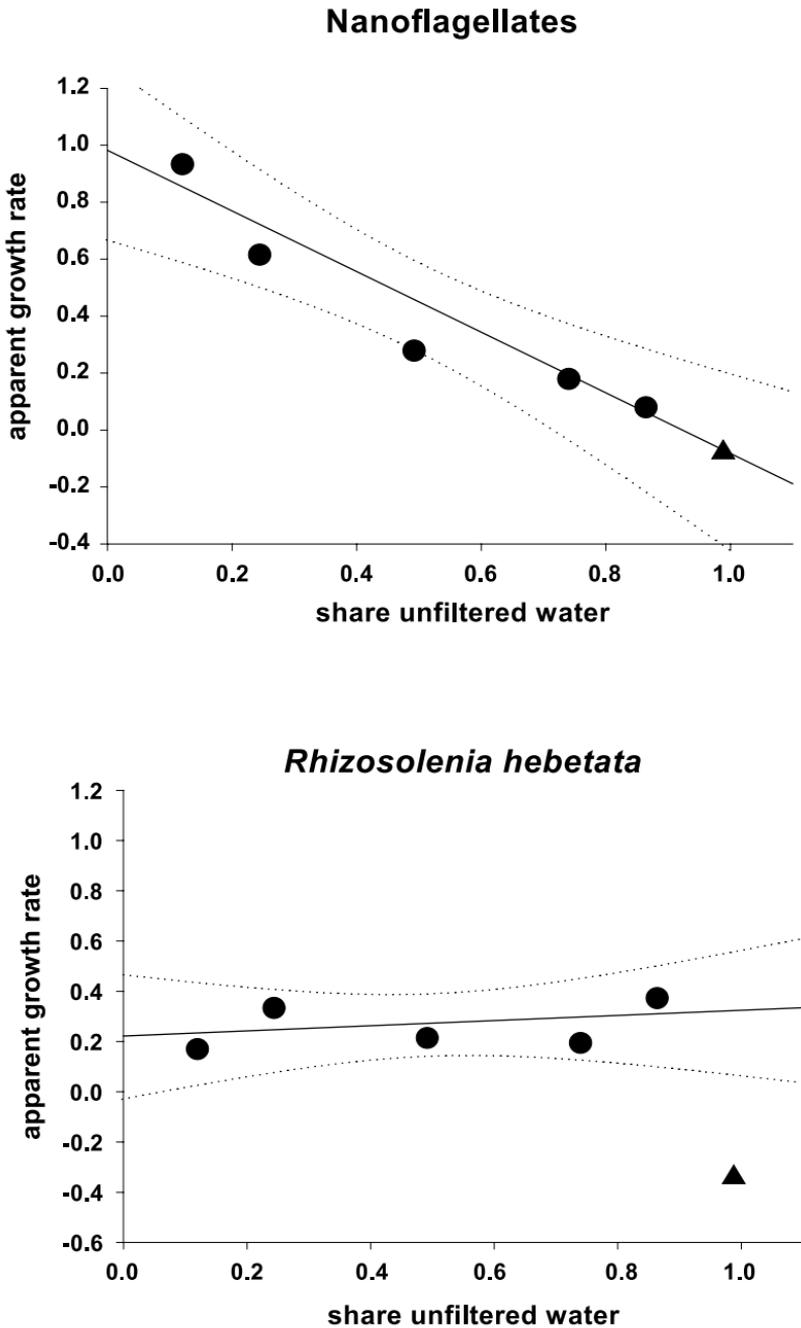


**Fig. 6.** Mesozooplankton grazing as a function of copepod abundance. Linear regression gave:  $y = -0.1 + 0.04 x$ ;  $r^2 = 0.66$ ;  $p < 0.01$ . Dotted lines represent 95 % confidence intervals.

tes had a significantly negative slope on the share of undiluted water ( $b = -0.98$ ; s. e. = 0.103;  $p < 0.01$ ; Fig. 7, upper panel). This indicates that nanoflagellates were strongly grazed by microzooplankton. There was almost no difference between the apparent growth rate predicted for an undiluted dialysis bag (no copepod grazing) and the growth rate in the mesocosm ( $-0.09$ ), thus indicating no significant grazing by copepods (Fig. 7). In the case of the large diatom *R. hebetata*, the slope of the regression was insignificant ( $b = 0.09$ ; s. e. = 0.056;  $p = 0.541$ ) indicating no grazing by microzooplankton. There was a conspicuous difference between the apparent growth rate for an undiluted dialysis bag and the apparent growth rate in the mesocosm ( $0.54 \text{ d}^{-1}$ ). This difference can be taken as an estimate of the strong copepod grazing rate of *R. hebetata* in the mesocosm. Among the species sufficiently abundant for counting, no single species was grazed upon by microzooplankton and copepods  $> 200 \mu\text{m}$  at the same time (SOMMER et al. 2005).

## Discussion

The combination of dilution and dialysis techniques allowed us to estimate the *in situ* phytoplankton gross growth rate and the impact of micro- and mesozooplankton on phytoplankton communities and algal species with a single and



**Fig. 7.** Apparent growth rates of unidentified nanoflagellates and of *Rhizosolenia hebetata* (black circles) in the dilution series in a copepod containing mesocosm. Triangles indicate apparent growth rates in mesocosm ( $r_{\text{meso}}$ ). The difference between the regression line and  $r_{\text{meso}}$  is an estimate of mesozooplankton (copepod) grazing ( $\gamma_{\text{cop}}$ ). Dotted lines represent 95 % confidence intervals.

easy to perform experiment. The combination of these two methods may overcome some of the problems associated with methods estimating apparent phytoplankton growth rates and microzooplankton grazing in bottle experiments as it includes less artificial manipulations. The usually slower growth rates of phytoplankton in bottles may be due to a variety of causes, including nutrient limitation and differences between phytoplankton growth in bottles and more open environments have been already discussed in detail (FURNAS 1982).

The combination of dilution and dialysis methods may also give estimates of mesozooplankton grazing in mesocosms experiments where control mesocosms without mesozooplankton can be established to control for additional losses of phytoplankton beyond micro- and mesozooplankton grazing. Techniques to estimate mesozooplankton grazing *in situ* are normally including tracers such as radioactive isotopes. However, it is not always possible to use radioactive tracer methods in the field. Additionally, radioactive tracer methods cannot as easily give simultaneous grazing rates for individual algal groups or species.

Our results of the marine mesocosms experiments clearly show that the above described method was able to yield ecological meaningful estimates of phytoplankton gross growth rates and micro- and mesozooplankton grazing. In experiment 1, ctenophores were influencing the abundance of meso- and microzooplankton via trophic cascade effects (Figs 1 and 2). These trophic cascade effects were also visible in the grazing impact of ciliates and copepods on phytoplankton which we estimated by dilution experiments incubated in dialysis bags. (Figs 5 and 6). The phytoplankton gross growth rates which were quantified within the same dilution experiments were similar between the treatments (Fig. 4). We expected this, as all treatments received the same amount of light and nutrients.

The estimates of meso- and microzooplankton grazing rates on different sized algae during experiment 2 were in concordance with estimates received by alternative methods and previous knowledge about size selective grazing in the marine pelagic zone (SOMMER & STIBOR 2002). According to our dilution experiments in dialysis bags large diatoms were not eaten by microzooplankton but by copepods, whereas small phytoplankton species were eaten by microzooplankton but not by copepods (Fig. 7; SOMMER et al. 2005). Alternative methods to obtain such simultaneous estimates of community- and group- or species-specific phytoplankton gross growth rates and meso- and microzooplankton grazing rates in a mesocosms experiment would normally involve a variety of different techniques.

There are limitations associated with the majority of methods estimating growth and grazing parameters in environmental microbiology. A common problem in dilution experiments is that grazers can grow or die during the in-

cubation and that these processes and per-capita feeding rate of grazers are different between undiluted and diluted samples, resulting in uncertainties in measured grazing rates (GALLEGOS 1989, EVANS & PARANJAPE 1992, DOLAN et al. 2000). However, these problems can be met by examining grazer populations during the experiments to assess possible artifacts in grazing rate estimates. Hence, counting the individual algal species in the dilution series by Utermöhl- or other related techniques would allow estimating micrograzer abundances within the same sample.

Whereas we concentrated on microzooplankton grazing influencing the growth response of phytoplankton within the dialysis bags one should have in mind that also a variety of other factors are of importance. The species composition (inherent growth potential), the standing crop in the bags (onset of diffusion limitation), external nutrient concentrations, temperature and irradiance will as well influence phytoplankton growth responses within the dialysis bags and a comparison of such experiments must consider these factors. The effective turnover time for nutrients in dialysis tubes will be dependent upon the type of dialysis tubing used and the shape of the dialysis bags. The surface to volume ratio of the bags and the degree of mixing around and within the bags will influence the diffusion dynamics across the dialysis membrane. However, detailed studies showed that the half life time for water in comparable dialysis bags were approximately 3 hours (FURNAS 1982) and measurements of nutrient dynamics showed that equilibrium with ambient water is reached between 4 and 8 hours (MURA et al. 1996).

Limitations of dilution methods are mostly defined by the assumptions on which the method is based and which are described in the introduction. Limitations will mainly evolve from shifts in possible density dependent nutrient-competition between phytoplankton due to dilution and a deviation from a linear grazing impact of microzooplankton on phytoplankton. This could result from different microzooplankton population dynamics within the dilution treatments in the dialysis bags (DOLAN et al. 2000). The question arises how large deviations from the assumptions can be before estimates of phytoplankton growth and grazing parameters become seriously incorrect. It will take some time before dilution related differences in phytoplankton competition or microzooplankton growth dynamics affect the apparent phytoplankton growth rates in the different dilution treatments. However, the shapes of the growth response of phytoplankton to dilution can be used to assess whether the assumptions of the method are sufficiently met. Non linear growth responses of phytoplankton to dilution will indicate that the assumptions are not valid in that specific case. Our examples demonstrate that the incubation of dilution experiments within dialysis bags in our mesocosms experiments led to linear growth responses of the phytoplankton in all treatments. It seems that the dilution experiments were performed for a short enough period before dilution re-

lated differences in competition between phytoplankton and/or microzooplankton population dynamics resulted in non linear growth responses of phytoplankton to dilution.

We have shown that the combination of dilution and dialysis techniques can be used to quantify simultaneously how grazer abundance, selective susceptibility to different grazers and nutrient supply will act on phytoplankton communities. Thereby, this method allows an estimate to which extend bottom-up and top-down forces act on phytoplankton dynamics *in situ*. The method is especially useful in mesocosms experiments where the exposition of dialysis bags is normally without problems and control mesocosms without mesozooplankton can easily be installed to separate between mesozooplankton grazing and other losses such as sedimentation. Therefore, the incubation of dilution experiments within dialysis bags may be an additional useful method to estimate important top-down and bottom-up fluxes within pelagic communities.

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