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FEATURE ARTICLE

Coupling between phytoplankton growth and microzooplankton grazing in dilution experiments: potential artefacts

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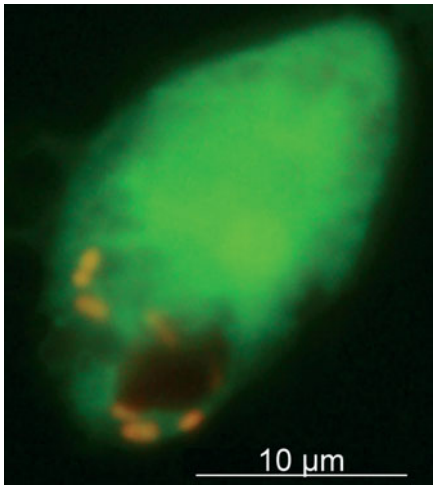
ABSTRACT: Phytoplankton distribution is relatively constant in large areas of the surface ocean. In order to maintain this apparent stability, phytoplankton production and losses have to be balanced. Indeed, growth (μ_o) and grazing (g) rates obtained simultaneously with the dilution technique are often tightly coupled. One problem with this approach is that growth and grazing are not independent in the ecological model on which the method is based (net growth rate = $\mu_o - g$). We evaluated to which extent this methodological artefact may influence the correlation between μ_o and g estimated using the dilution technique. Following a Monte-Carlo approach, we show that the methodological correlation can be substantial depending on: (1) the % error in the measurement of the state variable N_D (e.g. chlorophyll *a*) and (2) the range (± SD) of the μ_o and g considered. As long as the error of N_D is small (< 10 %), the measured correlation between growth and grazing closely reflects a true ecological relationship. For large errors, the dilution technique can yield a substantial correlation between both variables, regardless of their ecological relation. The influence of this methodological correlation decreases as the range of growth and grazing rate values increases. We developed a procedure to evaluate the ecological versus the methodological nature of the correlation observed between μ_o and g. The application of this procedure to a data set obtained from a coastal site revealed that the high correlation observed ($r_S = 0.881$, $p < 0.0001$) reflected a true ecological relationship.

KEY WORDS: Dilution technique · Phytoplankton growth · Microzooplankton grazing · Coupling · Monte-Carlo simulation



Epifluorescence microscope image of heterotrophic protist *Oxyrrhis marina* with ingested *Synechococcus* cells (orange).

Image: Jude Apple, Shannon Point Marine Centre

INTRODUCTION

Large areas of the ocean exhibit relatively constant phytoplankton biomass with little or no seasonal variation (Banse & English 1994, Banse 2002). This observation requires phytoplankton gains and losses to be balanced. Among the different loss processes, microzooplankton grazing is considered to be the main factor controlling phytoplankton biomass (Calbet & Landry 2004, Irigoien et al. 2005).

Different phytoplankton groups co-existing under the same environment display different growth rates

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(Furnas 1990, Strom & Welschmeyer 1991, Latasa et al. 1997). This group-specific growth rate diversity implies that fast- and slow-growing taxa must be grazed accordingly to keep phytoplankton biomass constant. Parallel measurements of phytoplankton growth and grazing have supported this view by showing a good correlation between both variables, either for phytoplankton measured as a whole (McManus et al. 2007) or for major phytoplankton groups (Burkill et al. 1987, Strom & Welschmeyer 1991, Latasa et al. 1997). In these studies, the experimental evidence was obtained with the dilution technique (Landry & Hassett 1982), the only approach allowing the simultaneous estimation of phytoplankton growth and grazing rates.

The dilution technique is based on the serial dilution of natural seawater aiming to generate a gradient of grazing proportional to the dilution gradient established in each incubation bottle (Landry & Hassett 1982, Landry et al. 1995). The apparent growth rate in each dilution treatment (μ_D), which is assumed to be exponential, is assessed from changes in a measured state variable of the phytoplankton population (chlorophyll *a* [chl *a*], carbon, cell counts, etc.) as:

$$\mu_D = \left(\frac{1}{t}\right)\ln\left(\frac{N_{D(t)}}{N_{D(0)}}\right) \quad (1)$$

where t is the incubation time and $N_{D(0)}$ and $N_{D(t)}$ are the chosen state variable of the phytoplankton population at the beginning and end of the incubation, respectively.

The apparent growth rate in each dilution treatment can be expressed as a function of the intrinsic growth rate (μ_o), fraction of unfiltered seawater or dilution gradient (f_D) and grazing (g) following Model A:

$$\mu_D = \mu_o - f_D \times g \quad (2)$$

Assuming that g and μ_o are constant, a set of equations can be built to describe changes in μ_D along the dilution gradient (f_D) (Landry & Hassett 1982). Linear regression analysis allows the estimation of μ_o and g, with their confidence limits, from μ_D and the known f_D. One problem with this approach is that phytoplankton μ_o and g are not mathematically independent, i.e. the errors in μ_o and g caused by an error in the calculation of μ_D are not independent.

The goal of this work was to unveil the nature of the correlation between μ_o and g in data sets obtained from dilution experiments and to estimate the extent to which the calculated correlation reflects a methodological artefact rather than a true ecological link. We pursued this objective with 2 different types of approximation: (1) experimental field work and (2) a simulation exercise. In both cases, we modified either μ_o or g in order to uncouple their dynamics, yielding 2 independent, paired series of μ_o and g. Our initial hypothesis stated that, when *in situ* ('true') μ_o and g are independent and follow independent dynamics, the correlation between μ_o and g estimated with the dilution technique ('observed') will not be significantly different from zero. 'True' rates assume no error, and 'observed' rates include a measurement error. We also checked the variability of the methodological/artefactual correlation as a function of (1) the range of the measured μ_o and (2) the number of μ_o and g pairs.

It is important to note that, in this study, we did not test the adequacy of the ecological model assumed in the dilution technique ($\mu_{net} = \mu_o - g$). Instead, we analyzed how the propagation of errors in the measurement of μ_{net}, inherent to this accepted model, affects the correlation between μ_o and g reported in the field.

MATERIALS AND METHODS

Set up and calculations. For the experimental field work, we uncoupled μ_o and g by changing the irradiance of the dilution experiment incubations compared to the original conditions. We carried out 14 pairs of dilution experiments with surface seawater from Blanes Bay (NW Mediterranean). One of the pairs was incubated at saturating irradiance levels that simulated *in situ* conditions (high light, HL), and the other at limiting irradiance (low light, LL). We expected μ_o to decrease under LL conditions (lower irradiance than *in situ*). Initially, we assumed grazing to be causally independent from phytoplankton growth rate and irradiance, although we are aware that microzooplankton grazing might respond to irradiance (Strom 2001).

For the simulation exercise, we generated noise-corrupted dilution experiments using the 14 estimates of μ_o and g obtained in the field. We then calculated μ_o and g in each simulated experiment (n = 14 experiments) and the Spearman correlation coefficient (r_S) between μ_o and g in the parallel series. We repeated this simulation exercise following a Monte-Carlo approach in order to obtain the statistics for the r_S (mean and SD). The correlation between 'true' μ_o and g was zero because grazing was kept constant, assuming the same level in all experiments. Thus, any correlation between 'observed' μ_o and g (hereafter the methodological r_S correlation) would be due to the fact that errors in estimated μ_o and g are not independent. We calculated this methodological correlation for different errors in the measurement of the state variable (e.g. for 10% error, meaning that the measurement of chl *a*, the chosen state variable in our case, is made with an error of 10%).

We also checked the variability of the methodological r_S as a function of 2 factors: (1) the range of the measured μ_o, i.e. how the range of the measured μ_o in our dataset affects the methodological r_S yielded by the simulation exercise; and (2) the number of μ_o and g

pairs (n). This allowed us to interpret the nature (methodological versus ecological) of the correlation between μ_o and g, estimated with the dilution technique, assuming an error in the state variable measurement ($\%N_D$).

Field work. Monthly dilution experiments were performed during 2005 with surface seawater collected 1 km offshore in Blanes Bay (the Blanes Bay Microbial Observatory, MO, 41° 40′ N, 2° 48′ E). Three additional experiments were carried out during the summer of 2007; one with surface seawater from the Blanes Bay MO and the other two with surface seawater retrieved 1 km offshore from Barcelona (41° 21′ N, 2° 10′ E).

The experimental design followed that described in Landry et al. (1995) and Latasa et al. (2005) and included further modifications based on Gallegos (1989) 'three points' rationale. Briefly, the experiment included 5 bottles filled with appropriate quantities of filtered seawater to reach 90, 80, 70, 60 and 50% dilution. Three replicates of 100% unfiltered (whole) seawater along with the dilution series (Bottles 1 to 8) were nutrient amended with f/2 Guillard nutrient medium and ammonium to a final concentration of 6 µmol l^{-1}. Urea and glucose were also added to a final concentration of 1.5 µmol l^{-1} and 1.0 µmol l^{-1}, respectively. Three additional bottles of 100% whole seawater without nutrient amendment were included in the experimental design (Bottles 9 to 11).

Bottles were incubated for 24 h in a temperature and light controlled culture room in the laboratory. The diel light/dark cycle was adjusted to the length of the day on which the experiment was carried out. We prepared 2 complete dilution sets in parallel; one incubated under HL (320 µmol photon m^{-2} s^{-1}), aiming to simulate *in situ* conditions, and a second set at LL conditions (10 to 15 µmol photon m^{-2} s^{-1}). We repeated this experiment on 15 occasions, covering most of the ecological conditions found at this coastal site throughout the year. One of the 15 experiments, conducted in December, did not work and was excluded from the analyses. We obtained 2 data sets of 14 μ_o and g pairs, the first reflecting *in situ* μ_o and g and the second reflecting the rates obtained under LL conditions. The non-parametric spearman correlation coefficient (r_S) between μ_o and g pairs was estimated for the HL and LL sets. We decided to use the non-parametric coefficient because of the low number of μ_o and g pairs available (14 pairs in our study).

Phytoplankton growth was assumed to be exponential and properly described by Model A (Eq. 2). Apparent growth rate of phytoplankton in each bottle was estimated following changes in pigment concentration, quantified using either High Pressure Liquid Chromatography (HPLC) or a Turner fluorometer. Sampling for pigment measurement, storage, extraction and quantification followed the protocol described in Latasa et al. (2005) and references therein.

Grazing was calculated as the slope of the Model A (Eq. 2) linear regression between μ_D and f_D in the nutrient amended set of incubation bottles. When non-linearities due to saturating feeding were detected, grazing was estimated as the difference between intrinsic growth rate (μ_n, y-axis intercept, phytoplankton growth rate in the nutrient amended bottles) and the mean μ_D estimated from the non-diluted triplicate bottles ($\mu_{net(n)}$) of the nutrient amended set. Intrinsic growth rate at ambient nutrient conditions (μ_o) was calculated as the mean μ_D estimated from the non-diluted unamended triplicate (μ_{net}) plus the estimated grazing rate ($\mu_o = \mu_{net} + g$). Grazing is assumed to be equal in nutrient amended and unamended bottles.

Monte-Carlo dilution experiments simulations. We first generated a set of 14 virtual dilution experiments applying Model A (Eq. 2), $\mu_D = \mu_o - f_D \times g$, where μ_o and f_D correspond to the 14 field experiments carried out at HL conditions and g was assumed to be constant and calculated as the mean of the same 14 field experiments. Thus, these virtual dilution experiments were built under the premise of independence between 'true' μ_o and g.

We then simulated dilution experiments giving errors to the state variable (chl *a* in our case). We considered a random error (εN_D) associated with the state variable measurement in each bottle (N_D) and assumed this error to be normally distributed around a mean of zero and a standard deviation (σ), ($\varepsilon N_D \sim N(0,\sigma^2)$). The SD of the error was given by the % error in the measurement of the state variable (i.e. the simulation of the 10% error means that our measurement of N_D is made with a 10% error). The condition that the noise-corrupted N_D measurement be positive further constrained the random error (εN_D). The μ_D in each simulated bottle incubated for 1 d was estimated following Eq. (3):

$$\mu_D = \ln\left(\frac{N_{D(t)}}{N_{D(0)}}\right) \quad (3)$$

The error in the measurement of the state variable can be incorporated in this expression ($\varepsilon N_{D(0)}$ and $\varepsilon N_{D(t)}$, error at the beginning and error at the end of each incubation). The error in μ_D ($\varepsilon\mu_D$) can be expressed as:

$$\varepsilon\mu_D = \ln\left[\frac{1+\varepsilon N_{D(t)}/N_{D(t)}}{1+\varepsilon N_{D(0)}/N_{D(0)}}\right] \quad (4)$$

where $\frac{\varepsilon N_{D(0)}}{N_{D(0)}} \sim N(0, 0.1^2)$ and $\frac{\varepsilon N_{D(t)}}{N_{D(t)}} \sim N(0, 0.1^2)$ for 10% error in the measurement of the state variable (see Box 1 for details).

Box 1. Formulation for a measurement error of 10 %

$$\varepsilon N_{D(t)} \sim N\left\{0, \sigma^2 = [0.1 \times N_{D(t)}]^2\right\} = N_{D(t)} \times N\left[0, \sigma^2 = (0.1)^2\right] \quad \text{(a)}$$

$$\varepsilon N_{D(0)} \sim N\left\{0, \sigma^2 = [0.1 \times N_{D(0)}]^2\right\} = N_{0(t)} \times N\left[0, \sigma^2 = (0.1)^2\right] \quad \text{(b)}$$

where N is a normal distribution with mean zero and variance σ^2. In our experimental protocol, N_0 in each bottle is calculated from N_0 in non-diluted bottles and the theoretical dilution factor as:

$$N_{D(0)} = N_0 \times f_D \quad \text{(c)}$$

We estimated $\varepsilon\mu_D$ as the difference between 'observed' μ_D (μ_{D_obs}, which includes the measurement error) and 'true' μ_D (μ_{D_true}, without error)

$$\mu_D = \ln\left[\frac{N_{D(t)}}{N_{D(0)}}\right] \quad \text{(d)}$$

$$\varepsilon\mu_D = \ln\left[\frac{N_{D(t)} + \varepsilon N_{D(t)}}{N_{D(0)} + \varepsilon N_{D(0)}}\right] - \ln\left[\frac{N_{D(t)}}{N_{D(0)}}\right] \quad \text{(e)}$$

$$\varepsilon\mu_D = \ln\left[\frac{N_{D(t)} + N_{D(t)} \times N(0, 0.1^2)}{N_{D(0)} + N_{D(0)} \times N(0, 0.1^2)}\right] - \ln\left[\frac{N_{D(t)}}{N_{D(0)}}\right] \quad \text{(f)}$$

$$\varepsilon\mu_D = \ln\left[\frac{N_{D(t)}[1 + N(0, 0.1^2)]}{N_{D(0)}[1 + N(0, 0.1^2)]}\right] - \ln\left[\frac{N_{D(t)}}{N_{D(0)}}\right] \quad \text{(g)}$$

$$\varepsilon\mu_D = \ln\left[\frac{N_{D(t)}}{N_{D(0)}}\right] + \ln\left\{\frac{[1 + N(0, 0.1^2)]}{[1 + N(0, 0.1^2)]}\right\} - \ln\left[\frac{N_{D(t)}}{N_{D(0)}}\right] \quad \text{(h)}$$

$$\varepsilon\mu_D = \ln\left\{\frac{[1 + N(0, 0.1^2)]}{[1 + N(0, 0.1^2)]}\right\} \quad \text{(i)}$$

We introduced this error term ($\varepsilon\mu_D$) in Model A (Eq. 2) and obtained Model A1 for the nutrient amended set (Bottles 1 to 8)

$$\mu_D = \mu_n - f_D \times g + \varepsilon\mu_D \quad (5)$$

and Model A2 for the unamended set (Bottles 9 to 11)

$$\mu_D = \mu_o - f_D \times g + \varepsilon\mu_D \quad (6)$$

where μ_n and μ_o are phytoplankton intrinsic growth rates in nutrient amended and unamended bottles, respectively.

At this stage, we had generated 14 virtual experiments with their corresponding μ_D for each simulated incubation bottle (11 bottles in each of the 14 experiments), noise-corrupted with a random error in the state variable. We then estimated μ_o and g from each of these virtual experiments and calculated the r_S correlation yielded by this set of 14 pairs of μ_o and g. This r_S is solely due to the fact that the errors in the estimation of μ_o and g caused by the error in the calculation of μ_D are not independent; it does not reflect an ecological relation between μ_o and g, but only the mathematical formulation of Model A assumed in the dilution method. The simulation exercise was repeated 1000 times to obtain the probability distribution of this methodological r_S correlation for a specific error in the measurement of the state variable. We ran the same simulation for different errors in the measurement of $N_{D(t)}$ and $N_{D(0)}$ (5 to 60 %) and calculated the mean and SD of the methodological r_S for each error level.

Finally, we analyzed the variation of the methodological r_S as a function of the number of μ_o values in the data set (n), the range of this series (measured as the SD) and a single parameter that combines the error in the measurement and the range of the μ_o values as expressed in Eq. 7:

$$L = \frac{\%N_D}{SD} \quad (7)$$

where $\%N_D$ is the relative error in the measurement of the state variable and SD is the standard deviation of the μ_o values.

RESULTS AND DISCUSSION

Experimental results

Growth and grazing rates results are shown in Table 1. Phytoplankton structure and associated seasonal dynamics are analyzed elsewhere (Gutiérrez-Rodríguez 2008). Here, we will focus on the comparison between rates under *in situ* HL and LL conditions.

Growth rates were higher under *in situ* HL than under LL conditions (Wilcoxon rank test, $p = 0.0001$, Fig. 1A). As expected, this trend was not statistically significant for grazing (Wilcoxon rank test, $p = 0.194$, Fig. 1B). Nevertheless, grazing tended to be higher under HL than under LL conditions.

Growth and grazing rates from HL experiments were significantly correlated ($r_S = 0.881$, $p < 0.0001$, $n = 14$, Fig. 2A). Under LL conditions, this correlation was lower but statistically significant ($r_S = 0.631$, $p = 0.016$, $n = 14$, Fig. 2B).

Simulation results

We calculated the probability distribution of the r_S between μ_o and g caused by the methodological approach, i.e. the methodological correlation. We obtained different probability distributions for the methodological r_S (mean ± SD) depending on the magnitude of the measurement error of the state variable. This probability distribution for a 10 % error in the measurement is shown in Fig. 3. The mean r_S increases with the measurement error (Fig. 4). When the measurement error is low, the methodological mean r_S between μ_o and g remains low (i.e. mean $r_S < 0.25$ for an error of 10 %). For larger measurement errors, the methodological mean r_S increases (i.e. error > 25 %; mean $r_S > 0.43$). This trend is due to the propagation of the error in the estimation of g that is caused by errors in the calcula-

Table 1. Results of all field dilution experiments carried out in Blanes Bay and near Barcelona under *in situ* saturating (high light, 320 µmol photon m^{-2} s^{-1}) and limiting irradiances (low light, 10 µmol photon m^{-2} s^{-1}). Intrinsic growth rate under *in situ* (μ_o) and nutrient amended conditions (μ_n) and grazing (g). Values are means ± SEM. p-value and r^2 from the Model A (Eq. 2) linear regression applied to estimate growth and grazing parameters

Day of the year	μ_o	g	μ_n	r^2	p
High light					
20	0.587 ± 0.058	0.154 ± 0.040	0.678 ± 0.038	0.492	0.024
46	0.825 ± 0.093	0.204 ± 0.072	0.898 ± 0.057	0.507	0.001
64	0.663 ± 0.062	0.036± 0.04	0.727 ± 0.039	0.052	0.254
130	1.64 ± 0.125	1.40 ± 0.125	1.75 ± 0.091	0.945	<0.0001
158	0.760 ± 0.117	0.029 ± 0.114	0.519 ± 0.106	0.006	0.859
180[a]	1.40 ± 0.049	0.710 ± 0.048	1.425 ± 0.036	0.972	0.002
184[a]	0.905 ± 0.07	0.62 ± 0.059	1.14 ± 0.055	0.9306	0.0079
187	1.91 ± 0.055	1.04 ± 0.032	1.93 ± 0.027	0.991	<0.0001
191[a]	0.923 ± 0.134	0.485 ± 0.120	1.31 ± 0.117	0.968	0.016
215	1.06 ± 0.069	0.572 ± 0.048	1.39 ± 0.032	0.959	<0.0001
256	1.16 ± 0.109	0.373 ± 0.051	1.57 ± 0.049	0.952	<0.0001
278	0.768 ± 0.046	0.354 ± 0.022	0.820 ± 0.022	0.947	<0.0001
309	0.651 ± 0.058	0.026 ± 0.050	0.737 ± 0.041	0.025	0.711
339	0.808 ± 0.107	0.417 ± 0.061	0.843 ± 0.059	0.779	0.004
Low light					
20	0.342 ± 0.064	0.321 ± 0.063	0.221 ± 0.028	0.80	0.003
46	0.114 ± 0.04	0.100 ± 0.017	0.163 ± 0.012	0.84	0.048
64	0.271 ± 0.394	0.032 ± 0.280	0.219 ± 0.278	0.06	0.370
130	0.913 ± 0.198	0.901 ± 0.195	1.06 ± 0.141	0.76	0.005
158	0.120 ± 0.073	0.148 ± 0.068	0.634 ± 0.068	0.21	0.248
180[a]	0.543 ± 0.057	0.276 ± 0.035	0.380 ± 0.024	1.00	0.001
184[a]	0.826 ± 0.085	0.423 ± 0.082	0.618 ± 0.074	0.81	0.039
187	0.520 ± 0.091	0.352 ± 0.056	0.407 ± 0.044	0.87	0.002
191[a]	0.212 ± 0.108	0.484 ± 0.075	0.381 ± 0.055	0.86	0.001
215	0.137 ± 0.068	0.119 ±0.056	0.114 ± 0.039	0.40	0.093
256	0.564 ± 0.074	0.276 ± 0.045	0.760 ± 0.071	0.69	0.075
278	0.203 ± 0.06	0.456 ± 0.060	0.268 ± 0.055	0.96	0.023
309	0.331 ± 0.094	0.228 ± 0.094	0.231 ± 0.066	0.51	0.071
339	0.785 ± 0.219	0.613 ± 0.213	0.603 ± 0.203	0.80	0.04

[a]Experiments carried out at the coast near Barcelona

tion of μ_D being introduced into the estimated μ_o with the same sign ($\mu_o = \mu_{net} + g$), i.e. an overestimation of g will cause an overestimation of μ_o. For small measurement errors, the error in g is also small. Because g is assumed to be constant for all experiments, this error is enough to randomize the rank of the g vector but does not have the capability of modifying the rank of the μ_o vector. Thus, for small measurement errors, the μ_o vector conserves its rank free from the influence of the g vector: the ranks remain independent.

As the magnitude of the measurement error increases, so does the error introduced in the estimated grazing. Then, the effect on μ_o is large enough to affect the rank of the μ_o vector, and the correlation between the rank of μ_o and g vectors increases. Thus, for large errors in the measurement of the state variable, the magnitude of the error in the 'observed' g is large; the random rank of the g vector influences the rank of μ_o and yields a high r_S correlation even with uncorrelated 'true' μ_o and g. In summary, a high methodological r_S is obtained when the measurement error leads to an error in the estimated grazing large enough to alter the rank of the μ_o dataset.

This explanation suggests that the variability of the methodological r_S should be sensitive not only to the magnitude of the measurement error (Fig. 4A), but also to the range (SD) of the values of μ_o. Fig. 4B shows the different sensitivity of the methodological mean r_S between three μ_o

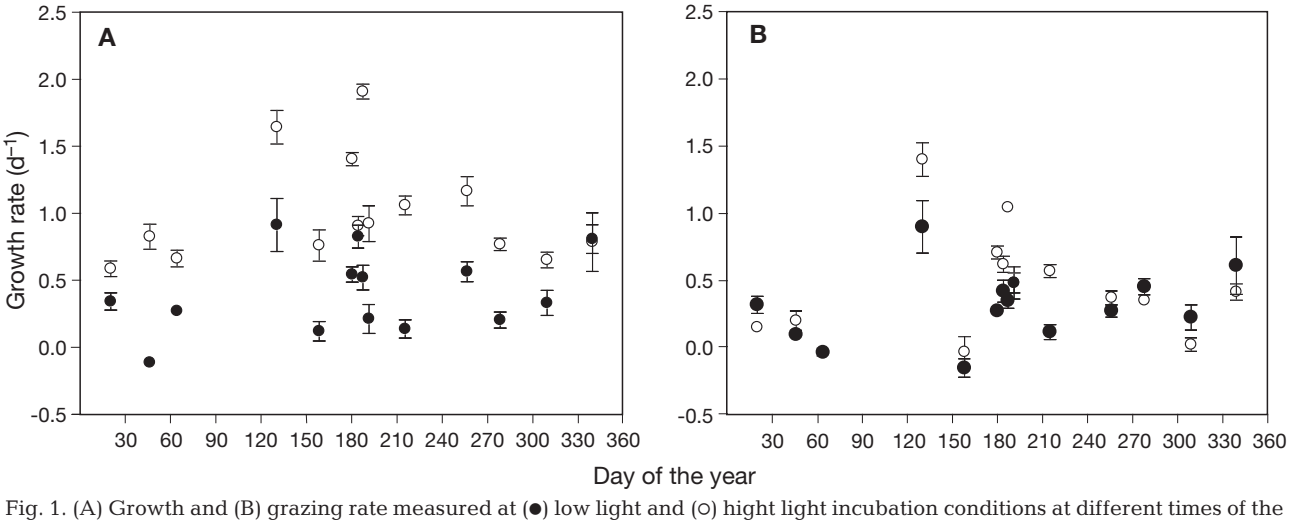

Fig. 1. (A) Growth and (B) grazing rate measured at (●) low light and (○) hight light incubation conditions at different times of the year. Error bars = SEM

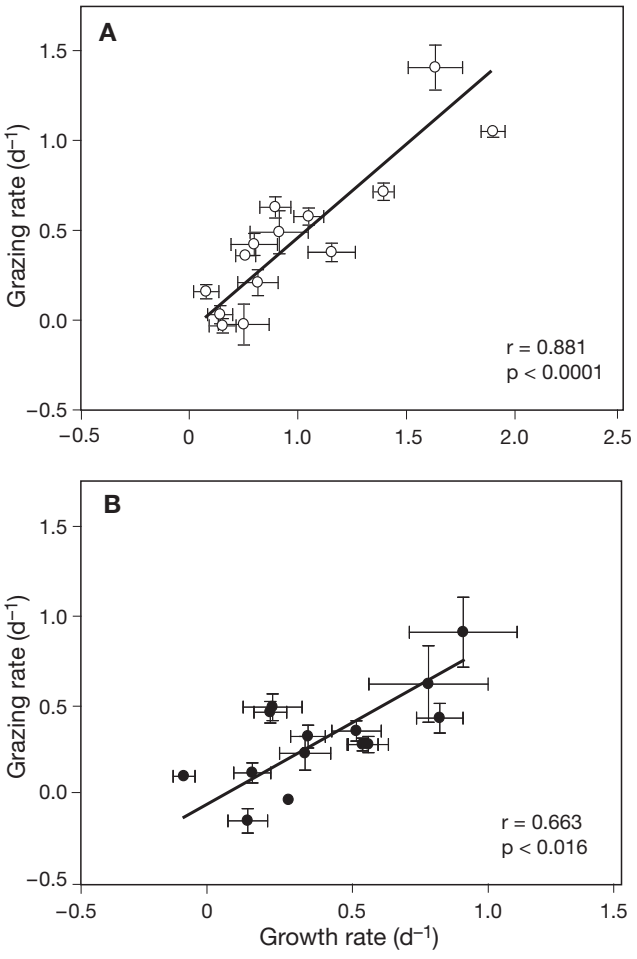

Fig. 2. The r_S correlation between intrinsic growth and grazing rate obtained under (A) high light and (B) low light conditions. Error bars = SEM

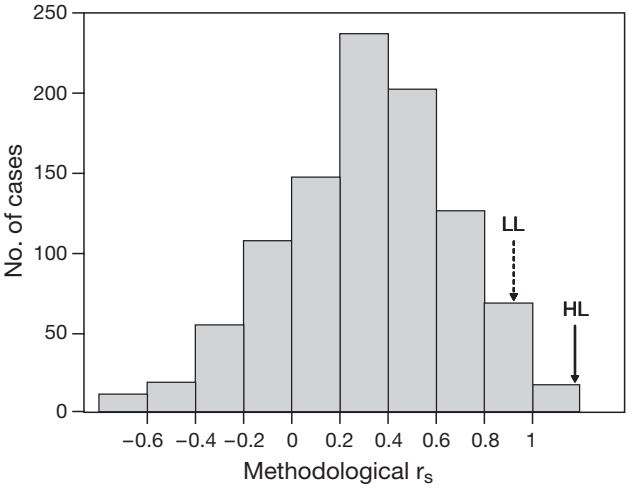

Fig. 3. Probability distribution of the r_S between growth and grazing, assuming a relative error of 10% in the measurement of the state variable (chl *a*). Solid and dotted arrows indicate the r_S calculated for our field experimental data set obtained from incubations under high light (HL) and low light (LL), respectively

datasets with different range (SD). The higher the SD of μ_o values, the more 'robust' is its rank relative to the measurement error variability. Thus, when dealing with phytoplankton μ_o and *g* obtained with the dilution technique, the correlation introduced by the method will be lower for data sets with higher variability (Fig. 4B). We identified 2 factors that determine to which extent the correlation observed between μ_o and *g* is real or an artefact of the dilution technique: the error in the measurement of the state variable and the range (SD) of μ_o.

We calculated a single parameter (L = $\%N_D$ / SD) that summarizes the combined effect of these factors. Fig. 5 captures the r-methodological variability as a function of the parameter L. In our simulation exercise, *g* is assumed to be constant and its variability (V_g) is due to the error in the measurement of the state variable (the numerator in the parameter L). The variability in μ_o ($V(\mu_o)$) accounts for the true variance of μ_o and the experimental error, and is quantified as the SD (the denominator of the parameter L). In summary, as long as V_g remains low compared to $V_x(\mu_o)$, the r-methodological will remain low. This conclusion is implicit in the following expression (see Appendix 1 for derivation):

$$V(\mu_o) = V(\mu_{net}) + V_g = \sigma^2(\mu_o) + \frac{\sigma^2}{3} + \sigma^2 \Big/ \Sigma\left(f - \bar{f}\right)^2 \quad (8)$$

where $\sigma^2(\mu_o)$ is the true variance of μ_o, σ^2 is the variance in μ_{net} caused by an experimental error, $\sigma^2/\Sigma(f-\bar{f})^2$ is the variance of *g*, *f* is the dilution factor and $\bar{f}$ is the average of the dilution factors used in the dilution experiments. The methodological correlation between μ_o and *g* will be small if $\sigma^2(\mu_o)$ is large compared to $\sigma^2/\Sigma(f-\bar{f})^2$.

The probability distribution of the methodological r_S plotted in Fig. 3 shows that some μ_o and *g* datasets might display a relatively high correlation even when the measurement error is low. This is also stressed by the 95% confidence intervals of the mean r_S shown in Fig. 4A. The SD is linked to the number of dilution experiments (n) included in the simulation. The larger the μ_o and *g* vectors (higher n), the more precise is the mean methodological r_S yielded by the simulation. The mean r-methodological does not change with an increase in the number of μ_o and *g* pairs, but the 95% confidence intervals of the mean become smaller (Fig. 5). This plot shows that the number of μ_o and *g* pairs (n) influence the 95% confidence intervals of the mean methodological r_S without affecting the mean itself.

Monte-Carlo simulation suggests that, in our field data set (μ_o = 1.01 ± 0.39 d^{-1}, mean ± SD, n = 14), the methodological correlation between μ_o and *g* is low (0.23 for a 10% error in the measurement of the state

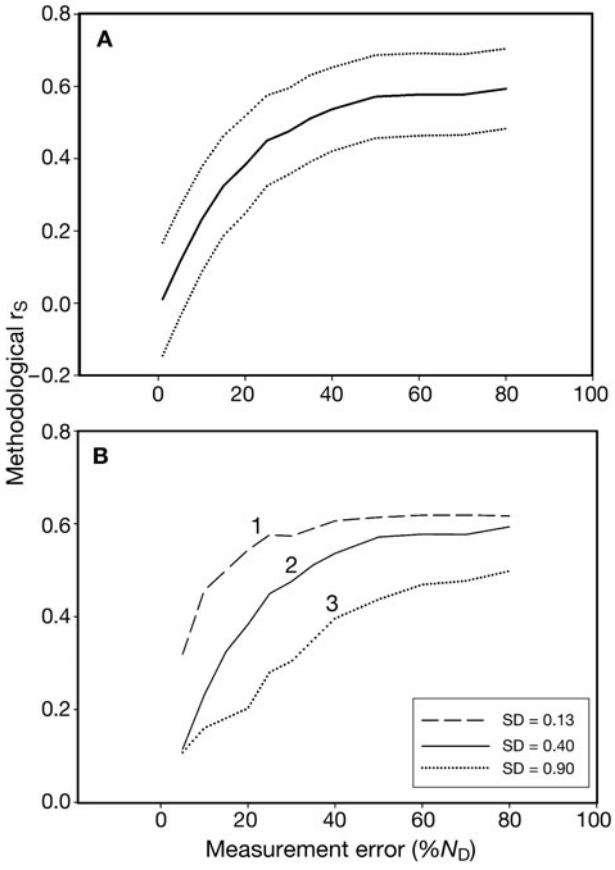

Fig. 4. Variability of the mean methodological correlation as a function of the % error in the N_D measurements (based on 1000 simulations). The line is composed of discrete values distributed evenly every 5% error until 30% N_D (i.e. 1, 5, 10…30%) and every 10% for errors > 30%. (A) μ_o and g pairs estimated in field experiments under high light (HL) conditions (n = 14, SD of μ_o = 0.40). Dotted lines are the 95% CI of the mean. (B) Effect of the ranges of μ_o (expressed as SD) on the methodological r_S. Curve 2 (solid line, n = 14, SD of μ_o = 0.40) is as in (A), Curve 1 (long dashed line, n = 14, SD of μ_o = 0.13) and Curve 3 (dotted line, n = 14, SD of μ_o = 0.90) are derived from μ_o and g pairs estimated in field experiments under HL conditions

variable). The r_S correlation between μ_o and g estimated at *in situ* HL conditions across the year in Blanes Bay was 0.881. The probability of obtaining such a high correlation with the dilution technique, if 'true' μ_o and g were independent, is < 0.1% assuming a reasonable measurement error of 10% (Latasa et al. 1996). Thus, the correlation we observed between μ_o and g in Blanes Bay reflects a 'true' ecological relation between these variables. Under LL conditions, the r_S between μ_o and g series estimated from parallel dilution experiments was 0.631. The probability of obtaining an equal or higher correlation, assuming that *in situ* μ_o and g were uncorrelated, is 6.6% for a 10% measurement error. Our results show that the steady state relation between μ_o and g can be modified by changes in light conditions, at least on a daily basis. In our simulation, it was essential that μ_o and g were independent. To assure independence, the μ_o vector maintained the variability observed in Blanes Bay throughout the year, while the g vector was assumed constant. However, the assumption of constant grazing might influence our results. We explored this possibility by running the same simulation exercise, but using randomly generated μ_o and g vectors, and we obtained the same results (Appendix 2).

SUMMARY AND CONCLUSIONS

We have demonstrated that the methodological errors in the estimation of μ_o and g can affect the correlation between μ_o and g obtained with dilution experiments that is often reported in the field. We have shown that the extent to which this correlation is due to the methodological procedure of the dilution experiments or to a true ecological relationship depends on (1) the error in the measurement of the state variable and (2) the range of μ_o and g values included in the correlation. As long as measurement errors are small (< 10%), the correlation introduced by the methodological procedure remains small, with most of the uncertainty coming from the usually limited number of experiments. Under these conditions, the frequently observed correlation between both variables is reflecting a true ecological link. For large measurement errors, the dilution technique can yield a substantial correlation between obtained μ_o and g, regardless of their ecological relation. This methodological correlation is

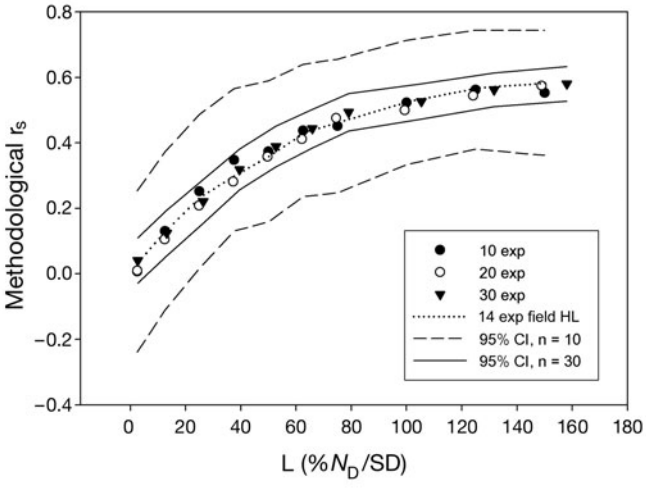

Fig. 5. Variability of the mean methodological r_S as a function of the parameter L for different data sets. Selected data points are indicated by (▼) n = 30, (○) n = 20 and (●) n = 10 simulations. ·····: field data set obtained at high light (HL) conditions (n = 14 experiments). The 95% CI for n = 30 (solid lines) and n = 10 (dashed lines) are shown

lower when the range of g and μ_o values is larger. In addition, we have shown that the number of μ_o and g pairs considered in the correlation analysis (n) determines the confidence interval of the mean methodological r_S yielded by the simulation, without affecting the mean value. We have performed the same simulation exercise using a parametric correlation coefficient (Pearson) and reached the same conclusions.

Finally, we provide an easy and systematic way to assess the mean methodological correlation of a given set of μ_o and g values from the range of μ_o values (SD) and the measurement error in the state variable ($\%N_D$) assumed by the scientist. This analysis allows us to affirm with confidence that the correlation between μ_o and g observed in Blanes Bay across the year reflects a true ecological relation. Moreover, this relation was affected by light. Irradiance affected μ_o more strongly than g, allowing the former to escape from grazing control, at least on a daily basis.

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Appendix 1. Evaluation of the effect of variable μ_o and g initial conditions on the change of the methodological mean r_S as a function of the measurement error

The basic Model A is

$$\mu_D = \mu_o - f_D \times g \quad \text{(A1)}$$

where μ_o is the intrinsic growth rate of phytoplankton, g is the grazing and f_D is the dilution factor (the fraction of undiluted seawater in each dilution bottle).

The parameter μ_{net} is the growth rate when $f = 1$ (whole seawater without nutrient amendement). It is estimated from triplicate measurements. Hence

$$\mu_{net} = \mu_o - g \quad \text{(A2)}$$

and

$$\mu_o = \mu_{net} + g \quad \text{(A3)}$$

The value of g is estimated from a linear regression of μ_{net} against f in enriched seawater. The assumption is that grazing is not affected by the enrichment. The obvious concern is the built-in correlation between μ_o and g in Eq. (A3).

Since the estimation of μ_{net} is based on measurements that are independent of the measurements used to estimate g, it follows that

$$V(\mu_o) = V(\mu_{net}) + V_g \quad \text{(A4)}$$

where $V_{(x)}$ is the variance of x. In other words, the variance of the sum is the sum of the variances when the variables are independent.

In the virtual experiment we envision, g is constant, and the variance of μ_{net} is calculated from

$$V(\mu_{net}) = \sigma^2(\mu_o) + \frac{\sigma^2}{3} \quad \text{(A5)}$$

where σ^2 is the variance of a growth rate measurement, and the factor of 3 accounts for the fact that the estimate is based on triplicate growth rate measurements.

The term $\sigma^2(\mu_o)$ accounts for the true variance of μ_o, and $\sigma^2/3$ accounts for experimental error. The variance of g is given by the expression

$$V_g = \frac{\sigma^2}{\sum(f - \bar{f})^2} \quad \text{(A6)}$$

where $\bar{f}$ is the average of the dilutions used in the dilution experiments. From Eq. (A4) it follows that

$$V(\mu_o) = \sigma^2(\mu_o) + \frac{\sigma^2}{3} + \frac{\sigma^2}{\sum(f - \bar{f})^2} \quad \text{(A7)}$$

We conclude that

$$\frac{V_g}{V(\mu_o)} = \frac{\frac{\sigma^2}{\sum(f - \bar{f})^2}}{\sigma^2(\mu_o) + \frac{\sigma^2}{3} + \frac{\sigma^2}{\sum(f - \bar{f})^2}} = \frac{1}{1 + \frac{\sigma^2(\mu_o) + \frac{\sigma^2}{3}}{\sigma^2 / \sum(f - \bar{f})^2}} \quad \text{(A8)}$$

V_g is less than $V(\mu_o)$. However, if most of the variance of μ_o is accounted for by the variance of g (exclusively due to the random error in the measurement), then μ_o and g will be highly correlated. The ratio $V_g/V(\mu_o)$ is negatively correlated with $\sigma^2(\mu_o)$ and positively correlated with σ^2. Thus, there will be little correlation between g and μ_o when $\sigma^2(\mu_o)$ is large and/or σ^2 is small.

Appendix 2. Mathematical demonstration of the influence of the methodological error (%N_D) on the correlation (r_S) between μ_o and g when Model A is applied

We demonstrated that the range of μ_o vector affects the methodological r_S (Fig. 5). However, the assumption of a constant grazing makes the rank of the g vector very sensitive to the measurement error variability. In addition, natural variability of growth and grazing are of similar magnitude, while we have eliminated the g variability in our approach. In order to evaluate the influence of the constant grazing initial conditions on our results, we performed the same simulation exercise, but using 2 independent vectors of μ_o and g generated with a random variability. This variability was kept within the range observed for these variables in the field work. This simulation exercise showed that the pattern of change in the methodological mean r_S as a function of the measurement error is independent of the variability of the g vector (Fig. A1). This is because the influence of the measurement error variability on the rank of observed μ_o and g, what we consider the methodological correlation, is lower when the variability of the true μ_o and g is higher.

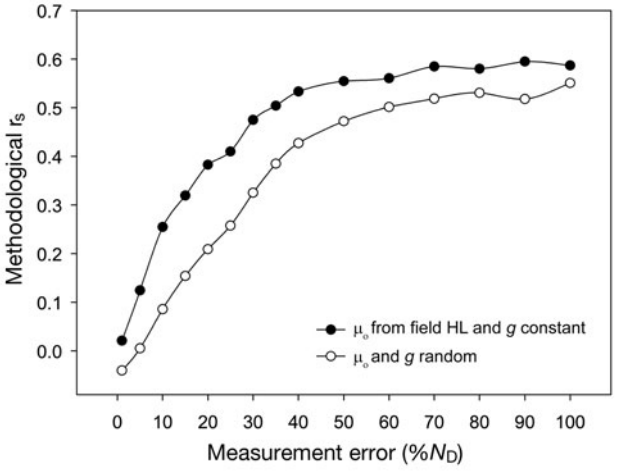

Fig. A1. Mean r_S correlation coefficient between μ_o and g produced by the methodological procedure as a function of the measurement error of the state variable. The 2 curves are associated with different initial μ_o and g vectors. ●: μ_o from field experiments with high light conditions and constant grazing; ○: curve is obtained using 2 independent vectors (μ_o and g) generated using a random variability with the range observed during field work

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