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***Interactive comment on “Scaling of growth rate and mortality with size and its consequence on size spectra of natural microphytoplankton assemblages in the East China Sea” by F. H. Chang et al.***

**Anonymous Referee #1**

Received and published: 28 December 2012

This study addresses the size-scaling of microphytoplankton growth rate and grazing rate due to microzooplankton using data from dilution experiments conducted in the East China Sea. Furthermore, the authors attempt to relate the size-scaling of microphytoplankton growth and mortality to the size-structure of microphytoplankton encountered in situ, in turn described by normalised-biomass size-spectra (NBSS). The main findings are that growth rate increases with cell size whereas mortality is size-independent. The authors also conclude that size differential grazing mortality drives the observed variability in the slope of the NBSS.

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Understanding the size-scaling of phytoplankton growth and mortality in the sea is an important topic, and relating these size-scaling relationships with the observed size-structure of phytoplankton assemblages represents quite a challenge. The data presented here may prove to be helpful in this respect. However, the paper as it stands has many serious shortcomings and its eventual publication could only be recommended if the authors undertake a really major revision. The changes needed are so substantial that it is fair to say an altogether new manuscript must be written. The key issues are: i) the observed positive size-scaling of growth rate in the studied cell size range is very difficult to explain mechanistically given our knowledge of phytoplankton physiology – therefore it may have been the result of an artifact of experiments or data analysis; and ii) given that the authors failed to observe size-dependence of mortality rate, size-differential mortality cannot be the driving force explaining the variability in the slope of the biomass size spectrum.

Below I structure my review as follows: i) writing, ii) data presentation and analysis, and iii) interpretation and conclusions.

### Writing

The writing is quite poor generally. It is obvious that the authors have not taken the time to review carefully the text before submission. Otherwise it is impossible to understand the number of spelling mistakes. As an example, I counted 8 spelling mistakes in the ‘Conclusions’ section alone, which is 12 lines long. Words such as ‘phytoplankton’, ‘assemblage’, ‘logarithm’ appear misspelled in various, sometimes imaginative, ways throughout the manuscript. Such lack of care is not acceptable: the ms in this form should never have been submitted to the journal. Once it was submitted, it should have been returned to the authors for a thorough correction before sending it to reviewers.

In addition to multiple spelling mistakes and poor grammar, the text suffers from lack of precision. Many procedures in the data analysis are not described clearly (e.g. the sections on path analysis and regression analyses are particularly confusing). References

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

to the literature are often inaccurate or irrelevant. A few examples follow:

Page 3, lines 1-2: 'Physiological constraints mainly base on the body size (Brown and Gillooly, 2003; Brown et al., 2000; Cermeño et al., 2006). This sentence is meaningless and the choice of references seems haphazard.

Line 28, page 12. Finkel 2004 is a lab study and does not report on the size-scaling of chl<sub>a</sub> content in the field.

Page 13 lines 12-14 The correct slope values in Maranon et al (2006) are the other way around (1.14 in coast and 0.96 in open ocean). Besides, and contrary to what the authors write (page 13, lines 11-12), this study does not compare locations with different irradiance (samples from the whole euphotic layer are used) but, rather, different nutrient availability.

#### Data presentation and analysis

Data presentation is excessively succinct. Only the combined size-scaling relationships for growth and grazing are shown. However, these data originate from individual dilution experiments – about which no information is given. How many of all dilution experiments conducted / size classes considered yielded significant slope values? What was the  $r^2$  in the regression analyses? At the very least, plots of  $\mu$  vs dilution factor from some representative experiments and size classes should be shown, and a table (or an Appendix) should be prepared containing the statistics.

Nothing is said about actual values for growth and mortality rates. After reading the ms, one does not know if phytoplankton in the region were growing at a rate of, say, 0.5 d<sup>-1</sup>, or 2 d<sup>-1</sup>. Rates reported in Figs. 4-6 are temperature-corrected (this should be clearly stated in the legends) and therefore cannot readily be interpreted in terms of real, in situ growth rates. This information should be given in a table. In addition, no description is made of the spatial variability in growth and mortality rates. For instance, was phytoplankton near the coast growing faster or slower than open-sea phytoplankton?

Full Screen / Esc

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Interactive Discussion

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No description is made of hydrographic conditions (temperature, mixing regime, nutrient concentration) in the different stations occupied. A Table is included as an Appendix but its contents are not even mentioned in the text. What is the relationship between those conditions and phytoplankton growth and mortality? And the same question goes for the biomass spectra: what is the relationship between hydrographic/nutrient variability and changes in the intercept and slope of the NBSS?

The manuscript should show the general hydrographic context and then present the spatial variability in measured rates (at least for the whole phytoplankton assemblage) as well as the parameters of the biomass spectra. The slope of the NBSS should be reported for each sampled station, and compared with data from the literature.

#### Interpretation and conclusions

Page 12, lines 10-12 When comparing their results with those reported in the literature, the authors must be aware of the differences in the cell size range considered. The present study focuses on the microphytoplankton size range – the cell biomass range goes from approximately 100 pgC/cell to 130000 pgC/cell, which is about 3 orders of magnitude in cell size. In contrast, the studies cited in this section, such as that of Chen and Liu (2010), consider much wider size ranges, from approximately 0.1 pgC/cell to 100000 pgC/cell (6 orders of magnitude). Chen and Liu (2010) found a positive size-scaling only in the small-to-intermediate cell size range, while the size-scaling was negative in the intermediate-to-large cell size range. It is this latter size range that is relevant for the present study, where also large cells are considered.

In the studies of Maranon et al and Huete-Ortega et al, again the size range considered was much larger and the overall conclusion was that the size-scaling of phytoplankton metabolic rate is isometric (e.g. there is no overall size-dependence of growth).

Therefore, none of the studies cited support the present paper's conclusion that growth rate increases with cell size in the microphytoplankton size range. In addition, virtually all laboratory studies published so far show that growth rates (or biomass-specific

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Interactive  
Comment

metabolic rates) decrease with increasing cell size in the microphytoplankton size range. A recent example can be seen in the study by Maranon et al. (2012) (Ecology Letters, DOI: 10.1111/ele.12052) which shows that the size-scaling of phytoplankton growth is in fact unimodal, which may be related to the size-dependence of nutrient uptake and use.

The argument used by the authors to explain the positive size-scaling of growth rates is not convincing (page 12 lines 27-31). In this argument, and assuming that chlorophyll content is a proxy for metabolic rate, one would expect to find a positive size-scaling in the relationship between mass- (or volume-) specific chla content and cell size in order to explain a positive size-scaling of growth rate (or biomass-specific metabolic rate). However, at most, there are reports of isometric relationship between chla content and cell size (e.g. lack of size dependence in chla content). No reports exist, to the best of my knowledge, of increasing chla content (per unit biomass or cell volume) with cell size (the package effect makes this possibility extremely unlikely). In addition, the comparison between field and laboratory studies made by the authors is misleading (page 12 lines 27-31). The study of Finkel (2004) showed a strong allometric relationship between cell size and chla content (that is, volume-specific chla content decreased markedly with increasing cell size) because the cultures used were growing under strong light-limitation, which enhances the package effect, particularly in larger cells.

In summary, the positive size-scaling of growth rate is rather hard to explain (for the size range considered by the authors) from a biophysical and physiological point of view. The authors should explore the possibility that this pattern may have resulted from some methodological artifact related to incubation, particle detection and volume estimation by the FlowCam, data analysis, etc.

A major conclusion of the study is that differential mortality of small versus large cells explains the variations in the slope of the NBSS. This argument is flawed: the authors did not find any sign of size-dependence in mortality. Within experiments, mortality is

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Interactive  
Comment

not related to cell size. Therefore, it is not correct to claim that (page 15) ‘relatively higher growth rate of small versus large individuals serves as a trigger for higher grazing mortality of small than large individuals’. In fact, the authors did not observe higher mortality in small cells compared to large cells. If they had, the relationships between mortality rate and cell size would not be flat (Fig. 5).

The authors base their argument on the observation that, when pooling together data from all experiments, there is a positive relationship between mortality and growth rate. However, the key distinction here is that this is variability between sites/stations, not within stations. It is to be expected that in those stations where growth is faster (slower), mortality will also be faster (slower). This has nothing to do with size-related effects and does not prove anything. In fact, the dilution method itself has a built-in tendency to show covariation between mortality and growth, because the first variable is the slope and the second variable is the intercept of the same regression line. The higher the slope, the higher the intercept is likely to be, and vice-versa.

The authors are confusing variability within the assemblage (changes in  $m$  or  $\mu$  along the cell size range) with variability between assemblages (waters with higher  $\mu$  are likely to have higher  $m$ , for all size classes). But in order to prove that size-differential mortality plays a role in the control of phytoplankton size structure, it has to be shown that size-related changes in mortality actually exist \*within\* a given assemblage. These changes do not exist, therefore size-differential mortality cannot be a factor explaining changes in the slope of the biomass spectra.

Finally, the range of hydrographic conditions and phytoplankton biomass encountered by the authors was relatively narrow. Chl  $a$  concentration varied only by a factor of 3–4. It is very difficult, with such a small range of variability, to detect a significant coupling between the size-scaling of growth/mortality and the biomass size-structure. The biomass-size structure observed at one particular time reflects the integrals of many gain and loss processes which have taken place over the previous hours/days. In contrast, the size-scaling relationships for growth and mortality result from instantaneous

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measurements and reflect only present conditions. Rather than attempting to link (on a station-to-station basis) the variability between the size-scaling of growth/mortality and that of biomass, a more realistic objective would be to assess whether the predominance of particular size-class (generally over the whole studied area) is likely to have been caused by bottom-up or top-down process, e.g. driven by growth or mortality.

#### Minor comments

Section 2.1. Sampling depths should be indicated. How many dilution experiments were conducted at each station and from which depths were water samples collected?

Page 11, lines 29 and rest of section. These sentences are quite confusing and difficult to follow.

Page 4, lines 24-25. 'high temperature favours the dominance of small phytoplankton' As shown recently (Maranon et al L&O 2012, 57, 1266-1278) temperature \*per se\* plays a very minor role in the control of phytoplankton size-structure. Small cells dominate biomass and productivity whenever resources (light and/or nutrients) are in small supply and carbon fixation rates are low, irrespective of temperature. The association between temperature and size-structure arises because temperature is often correlated with nutrient availability in the sea.

Table in Appendix. Clarify if these are mean values obtained from the euphotic layer. The term 'integrated' is misleading. Are the irradiance values also mean values for the photic layer? Do they refer to mean values over the light period? A value of '0' is reported for PAR in one occasion – this must be incorrect.

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Interactive comment on Biogeosciences Discuss., 9, 16589, 2012.

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