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## ***Interactive comment on “Food quality regulates the metabolism and reproduction of *Temora longicornis*” by R. Nobili et al.***

**Anonymous Referee #2**

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This manuscript presents results from a series of laboratory experiments on the grazing, egg production and respiration of *Temora longicornis*, fed on *Rhodomonas* sp. with different mineral ratios. The results are compared to the long-term data series on N:P ratios of zooplankton and seston from HOT and on *T. longicornis* abundance and seston N:P ratios from the coast of Norway. The main merit of the manuscript is, in my opinion, the large range in mineral ratios of *Rhodomonas* sp. which in principal allows a detailed analysis of the zooplankton response to the mineral food quality. Also, one must acknowledge the huge effort which must have gone to conducting the laboratory experiments.

However, the manuscript has some serious problems:

1) The use of different generations of the field collected copepods in experiments. It is

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well-known that copepod age, condition and feeding history can affect their metabolic rates (especially reproduction), but so does the time of the year (generation) when the copepods have been collected. For instance, copepods collected in late autumn or winter rarely produce as many eggs in cultures as do the copepods collected during the spring bloom. Maybe a generation effect could explain the peak respiration rates, as a similar effect was seen in unfed copepods (Fig. 2a)? The authors should produce a control experiment to demonstrate that the differences in copepod condition between the experiments did not bias the results. Maybe you have in each experiment included an optimal quality *Rhodomonas*?

2) Method description is incomplete, and it is thus not possible to evaluate the robustness of the results. The main questions are:

a. How were the different mineral ratios of *Rhodomonas* obtained? By using different dilutions of the media? Or different growth rates? Are you sure that the algal growth conditions didn't influence their biochemical content, production of exudates or something else than the mineral ratios? At least the cell size changed, which could be expected to influence the grazing rates.

b. What was the variation in food concentration, what was the food concentration in carbon, how were the grazing samples counter (coulter counter?), how many cells were counted per sample, what was the number of replicates in different treatments? I would suggest that the authors include a table which lists the date of the experiment, which treatments were included on that day (*Rhodomonas* ratios), which measurements were conducted and with how many replicates.

c. The statistics are messy, and it is difficult to keep track on all single regressions. One would simply like to know which treatments were significantly different from each other (in terms of copepod response, not cell size or concentration) and which factors influenced copepod responses. I would suggest one or another multiple correlation analysis or regressions, relating copepod responses to mineral ratios but also to food

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concentration and cell size. As copepod grazing rates vary 2-fold without any explanation, one wonders about the effect of cell size or concentration, which would logically be the first factors influencing copepod feeding; it is not sufficient to state that the concentration did not differ significantly between experiments.

3) Logic of the comparison to the field data is somewhat unclear to me, and the discussion is certainly over-interpreting the results. As annual biomass or abundance peaks of zooplankton are influenced by many factors not considered in the present analysis, the interpretation makes little sense to me. For instance, copepods are typically under a heavy predation pressure, and mortality would be expected to be the main factor controlling copepod biomass. Further, there is an abundance of studies investigating the effect of food quality and quantity in the field, and most of them conclude that food quantity rather than quality determines copepod success in the field. Also, copepods in the field do not feed on monospecific diets, but are known to be able to feed selectively, and to supplement their diet on e.g., microzooplankton with favorable mineral ratios. In the whole manuscript I'm missing references to the freshwater literature (e.g., Urabe Watanabe), where the connection between carbon growth efficiency and threshold ratios for mineral limitation in different zooplankton is well documented. Further, I would expect that the seasonal variation in mineral ratios of seston are large (and the changes in zooplankton species composition will fast induce seasonal changes also in zpl ratios), and typically larger than interannual differences.

Some more detailed comments:

- What was the algal growth rate? (row 90)
- The equation for calculation of SD is not necessary
- How much was filtered for C:N and P, and how many replicate filters?
- Algal carbon content and food concentration in carbon? Was that above the saturation of copepod feeding?

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- Please, plot the filtration rate and ingestion against cell volume and food concentration. The grazing rates should not vary this much with no reason.

- There is something wrong in the copepod size (row 245), *T. longicornis* females are around 1000  $\mu\text{m}$ .

- Rows 268-269: According your grazing data *T. longicornis* was feeding in these experiments.

- Results in general: Please plot respiration and egg production as a function of ingestion (in terms of carbon, nitrogen and phosphorus) and the GGE in nitrogen and phosphorus; these could provide more insights into potential limitations. I have difficulties to believe that the one peak egg production rate at 17:1 would be superior to the surrounding rates because of the mineral limitations. To strengthen the point you should show that the ingestion of nitrogen and phosphorus changes above and below the optimal ratio.

- Rows 369-370: Please compare your results (the rates, ratios etc.) to earlier studies, e.g., those of Kiørboe or Checkley.

- Discussion, chapter 4.3 onwards. For reasons listed above, I would leave all this out, and concentrate the manuscript on the experimental results.

For the reasons listed above, I unfortunately don't think that the manuscript should be published in its present form.

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