

## Response to Reviewer #1 Nobili et al. BG-2013-79

We thank Reviewer#1 for his/her detailed suggestions which have improved the clarity of the manuscript.

**Reviewer#1:** *Please revise according to the suggestions and comments below.*

*P 3204: are C:P and C:N ratios merely “indicators” of food quality or are they actually determinative of food quality? “with a food quality of 16N : 1P.” ?????? strange phrase. You should say “with and N:P ratio fo 16”. I can’t see how “food quality” can have units of N:P ratio!*

**Authors’ response:** Agreed. We have revised the wording of the abstract to include the determinative nature of nutrient ratios and revised the sentence ending with ‘with a food quality of 16N : 1P’. The two sentences now read ‘Food quality was determined from the stoichiometric N:P, C:N and C:P ratios of *Rhodomonas salina*.’ and ‘Maximum respiration (R), egg production rate (EPR), assimilation efficiency (AE), gross growth efficiency (GGE) and metabolic increment (MI) occurred when *T. longicornis* was fed on phytoplankton with an N:P ratio of 16.’

**Reviewer#1:** *“the Threshold Elemental Ratio (TER)” This is NOT how TER is defined according to existing stoichiometry theory. TER is the C:X ratio above which growth becomes X limited. At this point in abstract better to just say that 16:1 defines the optimum diet for Temora.*

**Authors’ response:** Agreed. We have revised the text of the Abstract accordingly. The sentence now reads ‘These data suggest that an algal composition of 16N:1P defines the optimum diet for *T. longicornis*’

**Reviewer#1:** *“phytoplankton organic N : P ratios can change on decadal timescales” They can also change on time scales much shorter than decadal!*

**Authors’ response:** Agreed. The sentence now reads ‘Field data show that phytoplankton organic N:P ratios can change on a range of timescales, and that a decadal increase in the food N:P ratio can co-occur with a shift to smaller sized zooplankton and a change in species abundance.’

**Reviewer#1:** *P 3205 Again, C:P and C:N ratios are not necessarily merely “indicators” of quality. But in many cases they are the DETERMINANTS of quality. That is, high C:P ratio means that that P is diluted into too much C, making it impossible to ingest P at a sufficient rate to maximize growth. But of course, as Tang and Dam (and others point out) often other things (such as PUFAs and amino acids and sterols etc) are co-varying with C:nutrient ratio.*

**Authors’ response:** Agreed. The sentence now reads ‘Hence, using N:P as a food quality determinant alongside the C:N and C:P ratios, should provide a more complete measure of the nutritional value of the food (Vrede et al., 2004).’

**Reviewer#1:** *“The global average elemental ratio of particulate matter of 106C : 16N : 1P, known as the Redfield ratio” Average where? Of particles IN THE OCEAN. Please specify. Also, you need to specify that these are atomic ratios.*

**Authors’ response:** Agreed. The sentence now reads ‘The global average elemental ratio (in atoms) of marine particulate matter of 106C:16N:1P, known as the Redfield ratio (Redfield, 1958),’

**Reviewer#1:** *“is considered to provide a balanced diet for copepods, with nutrient limitation occurring above and below this ratio.” Considered by whom? Citation? I thought that this study was the first to show this! If this property of food N:P is so well-known, then this manuscript should have low priority. (I don’t actually think anyone has provided strong evidence that copepods have maximal growth on food with N:P of 16).*

**Authors’ response:** Agreed. Indeed, as far as we are aware, this study is the first to show copepod nutrient limitation above and below an algal N:P ratio of 16. The sentence was meant to refer to how inorganic nutrient limitation affects the growth of algae. The paragraph has been amended.

**Reviewer#1:** *“phosphorous” !!!! here and elsewhere. Please spell correctly. “phosphorus”.*

**Authors' response:** Agreed. Phosphorus is now spelled correctly throughout.

**Reviewer#1:** "phosphorous constitutes less than 1 % of the total dry biomass of copepods"  
Not always the case. Some copepod stages can have %P higher than 1% . Please consult literature.

**Authors' response:** Agreed. This sentence now reads 'phosphorus constitutes ~ 1 % of the total dry biomass of adult marine copepods (Bamstedt 1986).

**Reviewer#1:** Overall comment on literature coverage in Introduction: It's very strange that very important papers on effects of stoichiometric food quality on zooplankton growth are not cited, including papers that call attention to unimodal shape of growth rate response to stoichiometric food quality.

For TER:

Urabe, J., & Watanabe, Y. (1992). Possibility of N or P limitation for planktonic cladocerans: an experimental test. *Limnol. Oceanogr.*, 37, 244–251.

For unimodal function: Plath, K., & Boersma, M. (2001). Mineral limitation of zooplankton: Stoichiometric constraints and optimal foraging. *Ecology*, 82, 1260–1269.

Boersma, M., and J.J. Elser. 2006. Too much of a good thing: on balanced diets and maximal growth. *Ecology* 87: 1325-1330.

**Authors' response:** Agreed. These references are now cited in the paper.

**Reviewer#1:** Methods. Duration of feeding rate incubations?

**Authors' response:** The feeding rate incubations were for 24h. This has now been added to the text.

**Reviewer#1:** "a speciifc seston N : P Threshold Elemental Ratio" again, this is a non-standard use of "Threshold Elemental Ratio"

**Authors' response:** Agreed. We have removed this term from the methods section.

**Reviewer#1:** Results P 3213 The writing is switching between past tense and present tense. Please stick to past tense.

**Authors' response:** Done

**Reviewer#1:** "a nutrient limited diet (9.8 : 1 N : P)" If 9.8 is a "nutrient-limited diet", then what is N:P of 22 (where growth is also low)? Just say "low N:P diet".

**Authors' response:** Agreed. The text has been amended.

**Reviewer#1:** Discussion P 3217: again, this is not how TER is classically used. TER is usually given as a C:X ratio and defines the onset of X-limitation. The authors need to deal with this issue and introduce a different concept of this optimal N:P effect rather than co-opting TER.

**Authors' response:** We agree that it is confusing to use TER, which has been used previously as the ratio between C and a nutrient (N or P), in the context here of an N:P ratio. We therefore define a new term following the same concept as the TER, the Threshold Nutrient Ratio (TNR<sub>N:P</sub>) which is the N:P ratio below which metabolism is limited by N and above which metabolism is limited by P.

**Reviewer#1:** "Therefore in this study, the poor nutritional quality of *R. salina* (i.e. lower and higher than 16.5 : 1) did not provide enough energy and speciifc nutrients essential for egg production, inhibiting growth." I don't see how the authors draw this conclusion. Feeding rate did not change with N:P, so energy intake was presumably unaffected by N:P. So, in that sense how did "the poor nutritional quality"

*fail to provide “enough energy”? (And, for that matter, how can “poor quality” provide anything, actually, much less energy? Sloppy writing here.)*

**Authors' response:** Agreed. The sentence has been amended to read 'Therefore, our study shows that *R. salina* characterised by an N:P ratio lower and higher than 16.5, does not provide the necessary balance of N and P for *T. longicornis* to achieve maximum rates of egg production.'