Response to Reviewer #2 Nobili et al. BG-2013-79

Reviewer #2 *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.*

Response We thank Referee #2 for his/her thorough review and agree that one of the merits of the manuscript is the large range of mineral ratios used to assess the physiological response of zooplankton to the quality of their food.

Reviewer #2 *However, the manuscript has some serious problems:*
1) The use of different generations of the field collected copepods in experiments. It is 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?

Response We don't have a control experiment where copepods collected at different times of the year were fed on *Rhodomonas* with an N:P ratio of 16. The reviewer acknowledges the effort which went in to conducting the culture experiments – it was not possible to undertake more than one N:P ratio experiment at a time. We considered using cultured copepods to ensure they were all in the same age group, however we decided against this based on evidence that the physiological responses of cultured copepods are reduced, and that inbred copepods are not representative of the in situ population. For example, after a few generations, cultured *T. longicornis* were found to have shrunk considerably in size and produced very few eggs, possibly due to lower genetic heterogeneity compared to natural populations (Klein Breteler & Gonzales 1988; Koski & Klein Breteler 2003).

The main variables affecting *T. longicornis* egg production in the field are body size/weight, food quantity, temperature and food quality (Castellani and Altunbas, 2006). For temperate latitudes any "generation effect" as described by the reviewer could be mainly ascribed to the effect of seasonal variation of body size (small animals in summer and large animals in winter) which is due in large part to seasonal changes in food quality and quantity (low in winter, high in spring, medium to low in autumn) and temperature. In our experiments, we constrained temperature (acclimatising the animals for days), food concentration (with carbon in excess) and body size (selecting adult females), the variable that was manipulated was food quality in terms of the N:P ratio.

As can be seen from Table 1, samples were collected between 29 June and 15 December, however as mentioned in the text and in Table 3, EPR experiments were only carried out on samples collected between October and December. The copepods were acclimated at the same conditions of food concentration and temperature for at least 2 days before the start of the feeding or respiration incubation.

Figure R1 shows EPR and R (fed and unfed) as a function of sampling date, with data separated between high (red N:P>17.5:1), low (blue N:P<15.5:1) and balanced (black 15.5:1>N:P<17.5:1) N:P ratios. The food quality experiments were randomised with respect to time, and there was no decline in rates when

all experiments together were tested as a function of the day of the year, or when experiments with similar food quality were tested as a function of the day of the year ($p \geq 0.2$).

The methods section 2.5, Table 1 and the discussion section 4.1 have been amended to clearly state when samples were collected and to put the case that there was no bias related to the time of year when the samples were collected.

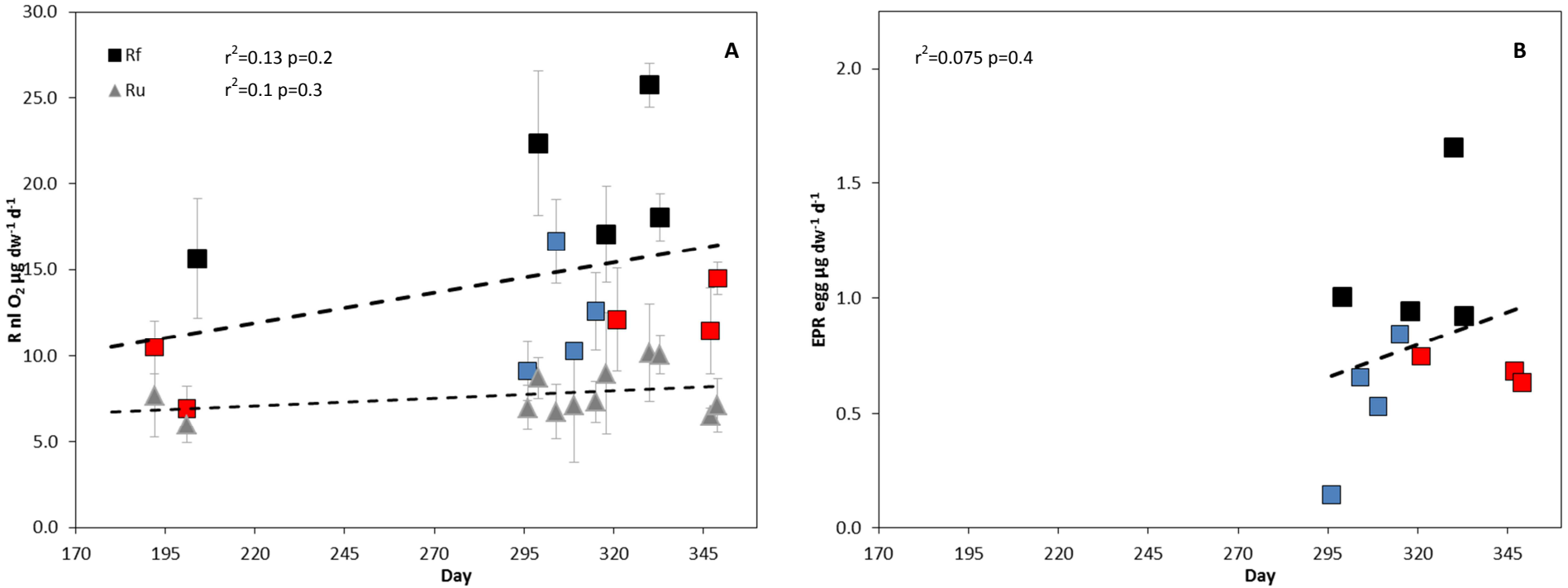

Figure R1: A) Respiration rates (R_f= fed; R_u= unfed) and B) EPR as a function of sample collection time (day of the year). The data are separated into high (red N:P>17.5:1), low (blue N:P<15.5:1) and balanced (black 15.5:1>N:P<17.5:1) *R. salina* N:P ratios. The black lines represent o.l.s. regressions, but are not significant. All measured rates are included.

The animals selected for R_u (R_f control) determination were not starved for any length of time prior to the incubation. Therefore the effect of N:P on R_u, we believe is due to the residual effect of the nutritional conditions (feeding history) experienced during 2 days of acclimation prior to the rate measurements. For instance it is well known that the reproduction rate of copepods is influenced by the food ingested during the previous 24 hours (Checkley 1980, Tester and Turner 1990). The Discussion text in section 4.1 has been amended to make this point.

Reviewer #2

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.

Response The different N:P ratios of *Rhodomonas* were obtained by changing the inorganic nutrient concentration of the media in which *R. salina* was grown. *R. salina* was first grown under three nutrient conditions – balanced (N:P=16), N-limited (N:P=3) and P-limited (N:P=60). The dilution rate for the three nutrient conditions was the same, in order to minimize confounding effects such as exudate production. For a particular experiment, copepods and seawater were collected from the North Sea. The seawater was then 0.2 µm filtered and used to dilute one of the three stock cultures to a particular N:P ratio and food concentration. Because of the large volumes of water that were needed in the feeding and respiration experiments it was not reasonable to do these experiments in artificial seawater. The diluted culture was then used for a feeding or respiration experiment. Although the feeding and respiration experiments were undertaken at low light, the algae still took up some nutrients from the filtered seawater during the 24h incubation. We had originally planned to use more than three nutrient ratios in the continuous cultures, but this was then found not to be necessary. At T_0 triplicate aliquots of 70-120 mL were filtered for C, N, P analysis and at T_{24} at the end of the feeding experiment, three control bottles were sampled (70-120 mL) and filtered for C, N and P analysis. The average change in *R.salina* N:P during the 24h incubation was a decrease of 1 N:P.

We have some biochemical data for *R.salina* which suggests that the effect we saw was related to N:P and not to cell biochemical composition. Polyunsaturated Fatty Acids (PUFA) were negatively correlated to C:N (r^2=0.52 p=0.004), but not correlated to N:P (r^2=0.15 p=0.17). Neither fed respiration (r^2=0.21 p=0.11) nor EPR (r^2=0.27 p=0.1) were significantly related to PUFA. In addition the total concentration of Saturated Fatty Acids (SAFA) was not related to N:P and was lower than the concentration of PUFAs in all experiments.

Table 1 shows that when all experiments are taken into account, cell size varied from a minimum of 98 to a maximum of 205 μm^3. However, within the limits discussed below, the total biovolume of food was kept constant, and cell size (μm^3) did not affect ingestion rates ($\mu gC\ \mu g\ dw^{-1}\ d^{-1}$) ($r^2$=0.05 p=0.5) (Figure R2A). In carefully reviewing the data, we realise that due to a malfunction of the coulter counter, on 11 and 20 July, cell size was determined from 2% lugols fixed samples, whereas all other measurements of cell size were made on fresh samples. Since we have no way of assessing the shrinkage of the cells due to the fixation process, we prefer not to show these two data in Table 1 in the revised manuscript.

The methods section 2.1, results sections 3.1 and 3.2, Table 1 and discussion section 4.1 have been amended, and Figure R2 added to Supplementary Material as Figure S1.For the sake of brevity, we have not included the fatty acids data in the manuscript.

Reviewer #2

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.

Response The *Rhodomonas salina* food concentration (cells mL^{-1}), measurements undertaken and replicates per measurement have been added to Table 1 alongside the nutrient concentrations per cell (pg $cell^{-1}$) to facilitate the calculation of nutrient concentrations in the food.

We aimed to supply a constant and saturating food concentration to *Temora* during all the experiments, however, due to a malfunction of the coulter counter requiring the initial culture dilutions to be based on flow cytometric data, on three occasions (11, 20 and 23 July), the food concentration supplied to the copepods was higher or lower than anticipated. This also meant that cell counts during feeding experiments on 11 and 20 July were undertaken on 2% lugols fixed samples. In the manuscript the ingestion and clearance rates obtained during 11 and 20 July were not further analysed and upon reflection we believe the ingestion and clearance data from 23 July should be treated in a similar manner. When all experiments are considered, the food concentration varied between 0.6 and 2.2 µgC mL^{-1}, when these three data are removed, food concentration varied between 0.9 and 1.4 µgC mL^{-1}. We argue below that retaining or removing these data makes no difference to the overall conclusions of the manuscript.

The grazing samples were counted with a coulter counter, the instrument carried out triplicate counts from each sample and we sampled each bottle three times. Only one *Rhodomonas* N:P ratio was used on any one day.

We have amended methods section 2.4.1 and results section 3.1 accordingly.

Reviewer #2

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 egressions, relating copepod responses to mineral ratios but also to food 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.

Response Multiple regressions are not appropriate because the response of R and EPR to N:P is not linear. We therefore split the N:P data into >16N:P and <16N:P, and gave all the significant relationships in Table 2. We have improved the text in the results section 3.2 to make the significant relationships clearer, and to stress that there was no significant relationship between either EPR or R and food concentration or cell size.

When data from 11, 20, 23 July are removed, the food concentration varied between 0.9 and 1.4 µgC mL^{-1}. Ingestion varied between 0.2 and 0.5 µgC µg dw^{-1} d^{-1} and was significantly related to food concentration (r^2 =0.34 p= 0.04) (Figure R2 B). This variation in ingestion rate (µg C µg dw^{-1} d^{-1}) was not related to EPR (r^2=0.2 p=0.17) or respiration rate (R_f r^2=0.24 p=0.12) (Figure R3).

Neither ingestion (r^2=0.05 p=0.5) nor clearance rates (r^2=0.05 p=0.5) were related to cell volume (Fig R2 A). Figure R2 has been placed in Supplementary Material as Figure S1.

EPR was not measured until 23 October 2011. However, R was measured on 11, 20 and 23 July, and since R was not related to ingestion, we decided to include these data in the analysis made in the manuscript. R is not related to ingestion either when these 3 data are removed (r^2=0.2 p=0.1), or when they are included (r^2=0.18 p=0.13). With the data included there are significant exponential relationships between R and N:P (R_f <16:1 r^2=0.88 p=0.005; R_f >16:1

r^2=0.92 p=0.006) (Table 2). With the data left out, the significant exponential relationship for R_f <16:1 (r^2=0.97 p=0.004) remains and the exponential relationship between R_f and N:P>16:1 becomes a significant linear relationship (r^2=0.79 p=0.017). The overall pattern of an optimum N:P is not affected. This is now better explained in the results sections 3.1 and 3.2 of the manuscript and Figure R3 is placed in Supplementary Material as Figure S2.

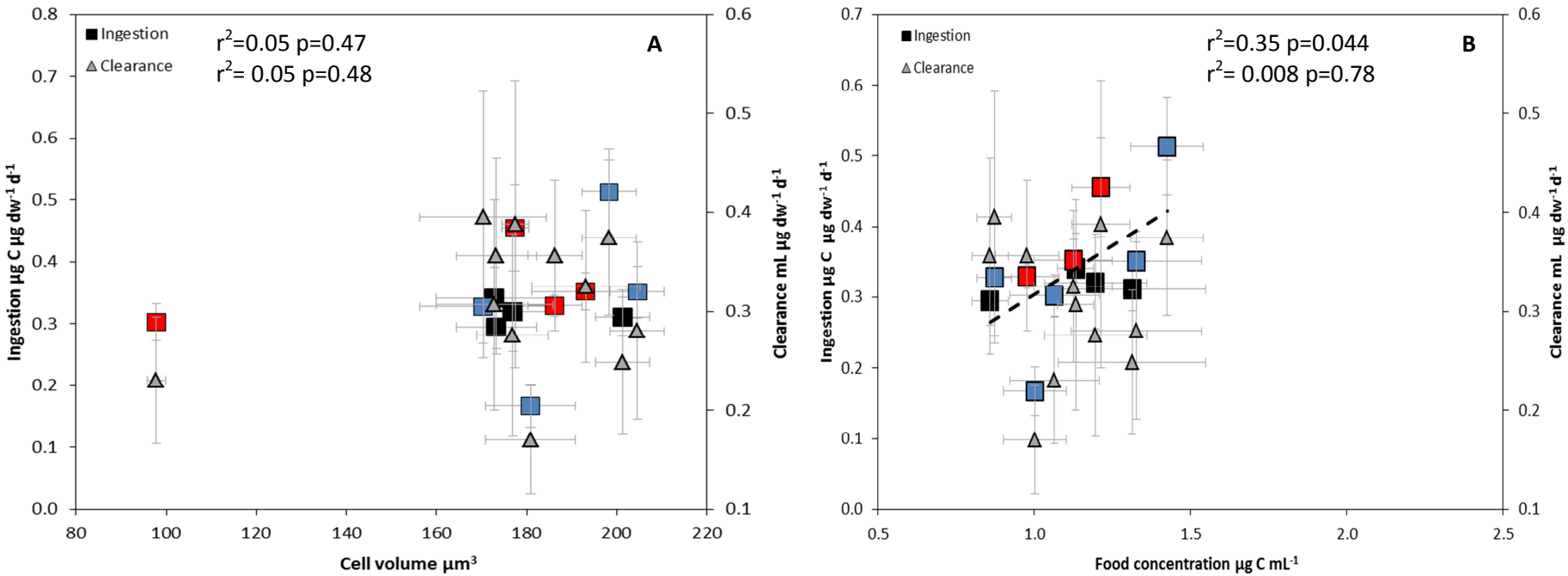

Figure R2: Ingestion and clearance rates as a function of A) cell volume and B) food concentration. The black line represents the significant regression between ingestion and food concentration (o.l.s). The ingestion data are coloured according to high (red N:P>17.5:1), low (blue N:P<15.5:1) and balanced (black 15.5:1>N:P<17.5:1) N:P ratios. Measurements of cell volume derived from 2% lugols fixed samples (11, 20 July) and of food concentration prepared when the coulter counter was broken (11, 20, 23 July) are not shown.

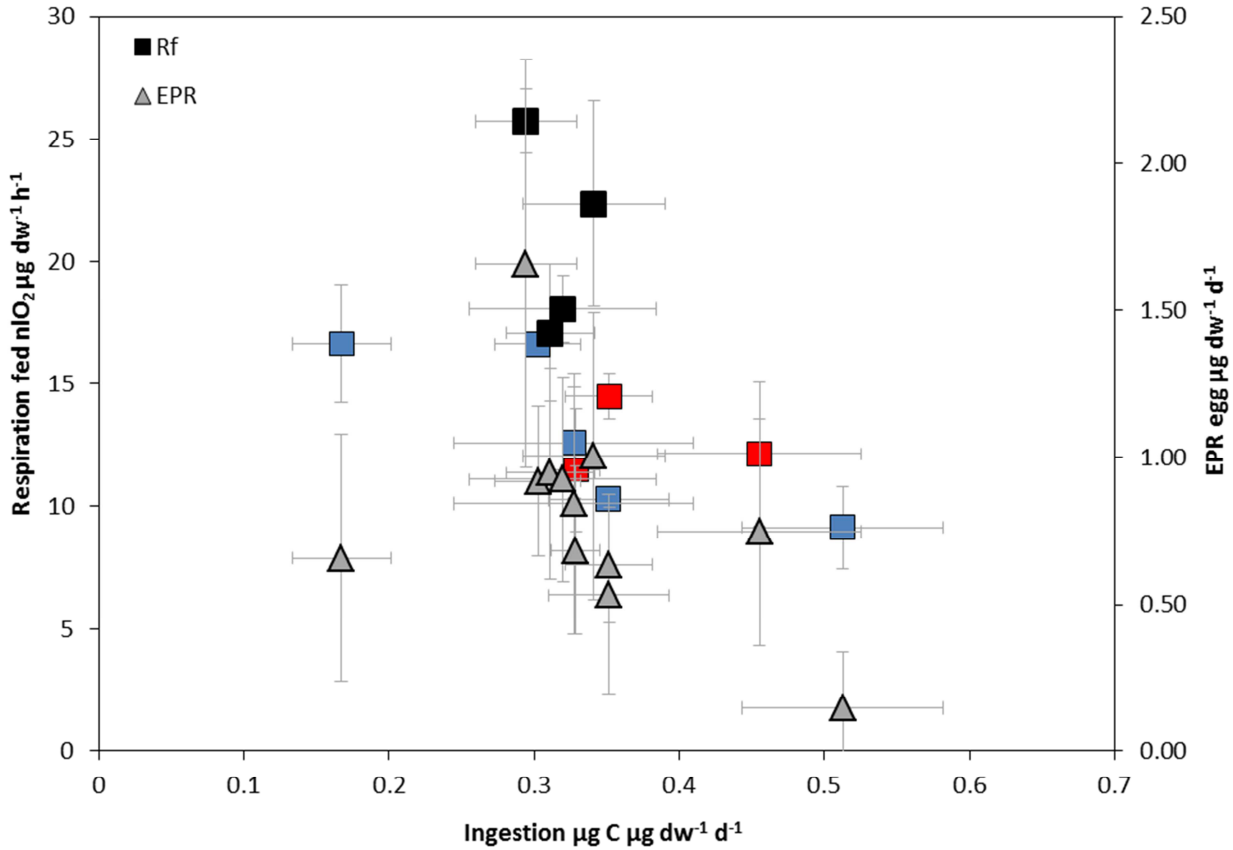

Figure R3: Measured respiration and EPR as a function of carbon ingestion (R vs ingestion r^2=0.24 p=0.12; EPR versus ingestion r^2=0.2 p= 0.17). Ingestion determined on 11, 20 and 23 July are not included. The respiration data are coloured according to high (red N:P>17.5:1), low (blue N:P<15.5:1) and balanced (black 15.5:1>N:P<17.5:1) N:P ratios.

Interestingly, although the malfunction of the coulter counter was beyond our control, when data collected on 11, 20 and 23 July are included (Figure R4 below), we were able to observe the effect of a ~5 fold range in ingestion rates on egg production and respiration rates. Egg production and respiration were independent of ingestion rate (Figure R4 C). Checkley (1980) showed that *Paracalanus parvus* egg production was independent of ingestion above a critical rate of ingestion. We suggest that by maintaining the food concentration at saturating levels, we achieved an analogous critical ingestion rate where ingestion was not related to respiration or egg production rates.

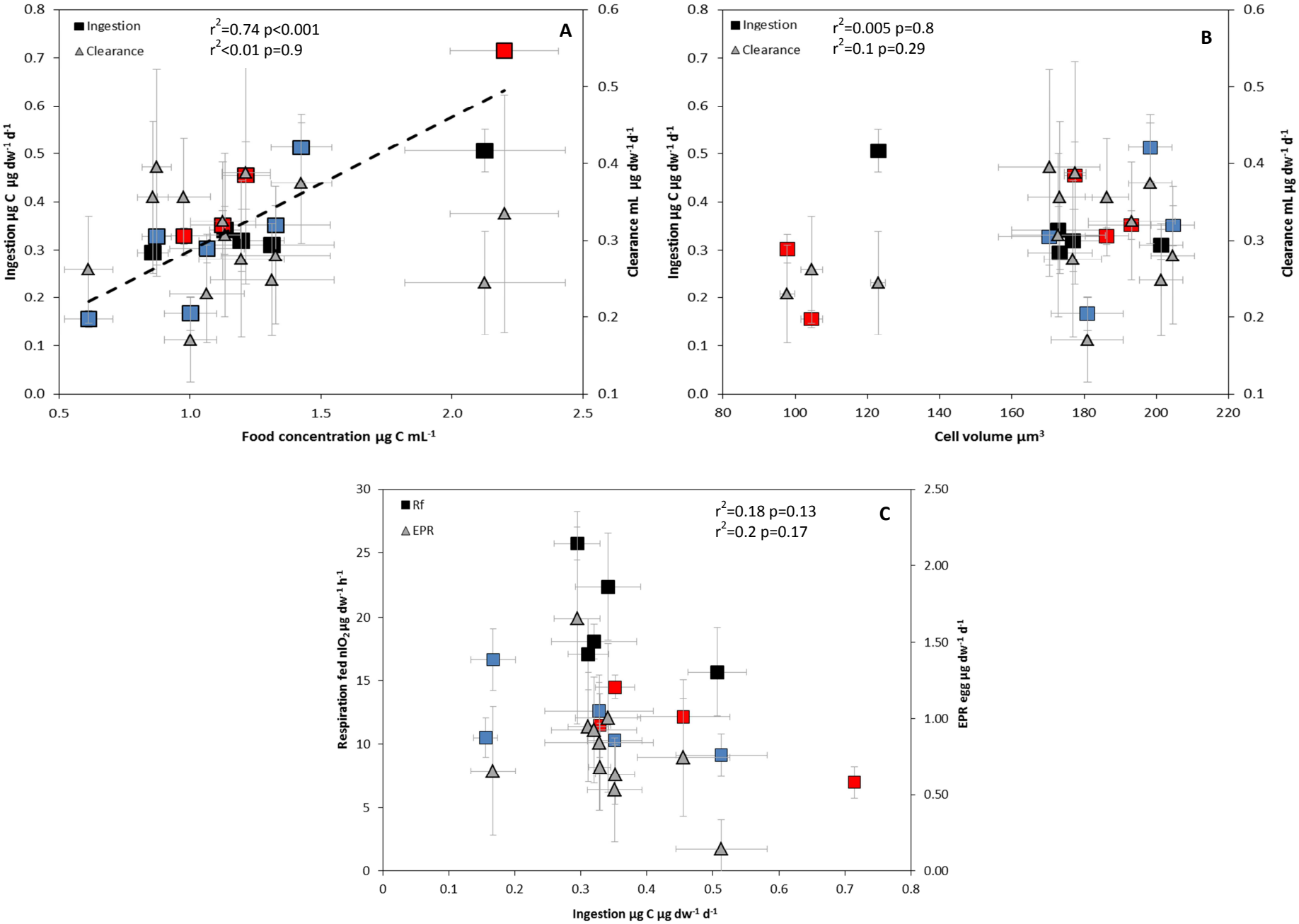
A
Ingestion
Clearance
r²=0.74 p<0.001
r²<0.01 p=0.9
Ingestion µg C µg dw⁻¹ d⁻¹
Clearance mL µg dw⁻¹ d⁻¹
Food concentration µg C mL⁻¹
B
Ingestion
Clearance
r²=0.005 p=0.8
r²=0.1 p=0.29
Ingestion µg C µg dw⁻¹ d⁻¹
Clearance mL µg dw⁻¹ d⁻¹
Cell volume µm³
C
Rf
EPR
r²=0.18 p=0.13
r²=0.2 p=0.17
Respiration fed nlO₂µg dw⁻¹ h⁻¹
EPR egg µg dw⁻¹ d⁻¹
Ingestion µg C µg dw⁻¹ d⁻¹

Figure R4: A) Ingestion and clearance rates as a function of food concentration. The black line represents the significant regression between ingestion and food concentration (o.l.s). B) Ingestion and clearance rates as a function of cell volume, C) Respiration and EPR as a function of carbon ingestion. The ingestion data are coloured according to high (red N:P>17.5:1), low (blue N:P<15.5:1) and balanced (black 15.5:1>N:P<17.5:1) N:P ratios. All measured data are included, except in panel B, where volume estimated from fixed samples on 11 and 20 July are not shown.

Reviewer #2
3a) 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.

Response We agree that mortality is the main factor controlling the spatial distribution of copepod biomass in the global ocean, but what we're discussing in this paper are changes in biomass over almost 20 years. We have no indication that these changes in biomass are due to changes in top-down control. In fact, at HOT we have circumstantial evidence that, if anything, would suggest that top-down control is counteracting the subsequent peaks in the biomass of size fractions. This is because the peaks progress from large to small copepods, while if top-down control were important the expected progression would be from small to large copepods, with a timing that was controlled by the generation time of each size class, while the timing that we see is clearly driven bottom up by the changes in nutrients (which are thought to originate from changes in circulation). Our laboratory results provide a mechanistic explanation for these changes, and the timing, the progression from large to small zooplankton, and the occurrence of biomass peaks in response to a continuous trend in nutrient stoichiometries are all consistent with our mechanistic explanation. We emphasize in the paper that "consistent with" is not the same as proof, but based on the supporting evidence we find that the food quality effects we find in the laboratory could have a measurable effect on field populations, and we think this part of the manuscript is important to stimulate targeted field studies that could provide this proof.

Reviewer #2
3b) 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.

Response We disagree with the Reviewer. Since the effect of food quantity and quality tend to co-vary, unless one does a controlled laboratory experiment, it is impossible to address this issue properly. The increasing number of studies investigating the effect of food quality on secondary production is evidence of the current lack of knowledge on this topic (Jonasdottir, 1994; Jonasdottir et al., 1995, 2009; Kleppel et al., 1998; Plath and Boersma, 2001; Dam and Lopes, 2003; Hessen, 2008; Koski et al., 2010; Schoo et al. 2012). Koski et al. (2010) evaluated the effect of food quantity and quality on egg production of North Sea copepods. In their study, multiple regression analysis did not detect an influence of the food quality variables measured (Chl a, phytoplankton pigments, POC, PON, fatty acids and sterols), however they concluded that cultured *T. longicornis* egg production was limited by the quality of food collected in May and June.

Temperate and higher latitude zooplankton are known to maximise population growth during a window of reproductive opportunity; these coincide with food quantity and quality peaks caused by phytoplankton blooms. Our experiment aimed to assess how changes in food quality, without changes in food quantity, could affect copepod biomass. It was therefore very relevant to assess field data for long term changes in food quality, at high and relatively constant food concentrations (spring bloom) alongside data of copepod biomass. This first step highlights the need for a full statistical analysis of field data to assess all the co-varying environmental factors, but hints that at certain crucial times of the year, food quality may be as important as food quantity. We agree that copepods

in the field do not feed on monospecific diets, and that at times of unpalatable phytoplankton (e.g. Phaeocystis blooms), *T. longicornis* in the North Sea can switch to a diet mainly composed of ciliates. However, this does not negate our first assessment of a link between *T. longicornis* abundance and the N:P ratio of North Sea seston (which includes both autotrophs and heterotrophs > 0.7 µm).

Reviewer #2
3c) 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.

Response
We agree, and have incorporated the paper of Urabe and Watanabe (1992) into the text of the Introduction and Discussion.

Reviewer #2
3d) 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.

Response In fact, interannual variability is more than an order of magnitude larger in both the HOT and North Sea data (variance of annual averages of 44 mol/mol at HOT (n=17) and 8.6 mol/mol at the North Sea Arundel station (n=21)) than seasonal variability (variance of a climatological year with averages for each month (n=12) of 1.78 mol/mol at HOT and 0.68 mol/mol at the North Sea station). We agree with the reviewer that this is an important point which we did not explain in the manuscript. As it is, it makes our analysis less subject to potential biases, such as interannual variability in the timing of the spring bloom, and we have clarified this in the manuscript.

Reviewer #2 *Some more detailed comments:*
- What was the algal growth rate? (row 90)

Response The growth rate of *R. salina*, is equivalent to the dilution rate of the continuous culture (i.e. 540 mL / 1.5 L given in the Methods section 2.1). The explicit growth rate of 0.36 cell division day^{-1} has now been added to Methods section 2.1.

Reviewer #2
- The equation for calculation of SD is not necessary

Response Agreed, this has now been deleted.

Reviewer #2
- How much was filtered for C:N and P, and how many replicate filters?

Response Triplicate samples (70 to 120 mL) of the culture used in the feeding experiments (T_o and T_{24}) were filtered for C, N and P analysis of *R. salina.* This has been added to section 2.2 in the Methods.

Reviewer #2

- Algal carbon content and food concentration in carbon? Was that above the saturation of copepod feeding?

Response We used an algal concentration of 5 x 10^6 µm^3 ml^{-1}, as this reflects bloom conditions in the North Sea (Jonasdottir and Kiorboe, 1996). This should also reflect saturating food concentration since O' Connors (1980) noted that maximum ingestion rates of *T. longicornis* occurred at food concentrations exceeding 5 to 10 x 10^6 µm^3 ml^{-1}, when fed on natural food assemblages. Specifically his results show that for cell sizes ranging between 6-8 µm, saturating conditions were reached at an algal concentration of 5 x 10^6 µm^3 ml^{-1}. In addition, throughout our experiment the food concentration in carbon units was always >0.5 µg C ml^{-1} (See revised Table 1). Previous studies suggest that an amount of 0.4 µg C ml^{-1} is saturating (Klein Breteler et al., 1995; Koski et al., 2011). Therefore we believe that the algal carbon content and food concentration in carbon was always above the saturation of the copepod feeding. The Discussion section 4.1 has been amended.

Table 1. Revised. Stoichiometric ratios (N:P, C:N & C:P mol mol^{-1}), carbon, nitrogen and phosphorus content (pg cell^{-1}), cell volume (µm^3) and concentration (cell mL^{-1}) of *R. salina* and number of replicates for each respiration (R), egg production (EPR) and feeding experiment (F). Data are the means of measurements taken at the beginning (T_0) and at the end (T_{24}) of the feeding incubation ± SD. NA = data not available.

	Stoichiometry			Nutrient measurements			Size	Food conc	Replicates		
Date	N:P	C:N	C:P	C pg cell^{-1}	N pg cell^{-1}	P pg cell^{-1}	Cell µm^3	cells mL^{-1}	R	EPR	F
29/06	14.8:1 ± 0.7	6.2:1 ± 0.2	92.2:1 ± 2.9	35.1 ± 0.7	6.5 ± 0.3	1.0 ± 0.06	98 ± 2	30369 ± 4025	NA	NA	1
11/07	10.2:1 ± 0.7	7.5:1 ± 0.3	77.4:1 ± 3.9	38.8 ± 1.0	6.0 ± 0.2	1.3 ± 0.12	NA	15815 ± 2355	5	NA	2
20/07	22.8:1 ± 0.5	7.4:1 ± 0.2	168:1 ± 3.4	45.2 ± 0.9	7.1 ± 0.1	0.7 ± 0.01	NA	48675 ± 4447	5	NA	2
23/07	15.6:1 ± 0.8	8.3:1 ± 0.2	128:1 ± 5.6	45.0 ± 0.6	6.3 ± 0.1	0.9 ± 0.05	123 ± 2	47298 ± 6782	5	NA	2
23/10	9.6:1 ± 0.6	9.9:1 ± 0.7	94.3:1 ± 4.1	55.2 ± 2.0	6.5 ± 0.5	1.6 ± 0.05	198 ± 6	25841 ± 1864	7	3	2
26/10	15.5:1 ± 0.4	5.9:1 ± 0.2	92:1 ± 2.0	34.7 ± 0.6	6.8 ± 0.2	1.0 ± 0.02	173 ± 13	32704 ± 1592	5	2	2
31/10	14.2:1 ± 1.0	6.7:1 ± 0.2	90.9:1 ± 4.3	33.8 ± 0.4	5.9 ± 0.1	1.0 ± 0.07	181 ± 10	29619 ± 2965	8	2	2
05/11	12.3:1 ± 0.6	7.9:1 ± 0.2	94.2:1 ± 3.5	50.9 ± 0.8	7.5 ± 0.2	1.4 ± 0.06	205 ± 6	26041 ± 4067	4	3	1
11/11	13.3:1 ± 0.7	5.8:1 ± 0.3	76.4:1 ± 2.1	33.5 ± 0.6	6.8 ± 0.3	1.1 ± 0.04	170 ± 14	26037 ± 1588	6	4	2
14/11	17.3:1 ± 0.8	8.1:1 ± 0.2	138:1 ± 4.8	46.8 ± 0.6	6.8 ± 0.1	0.9 ± 0.04	201 ± 6	28059 ± 5035	7	2	1
17/11	20.3:1 ± 0.5	5.3:1 ± 0.1	108:1 ± 1.6	38.4 ± 0.5	8.5 ± 0.2	0.9 ± 0.01	178 ± 3	31645 ± 2366	5	4	1
26/11	16.5:1 ± 0.5	5.2:1 ± 0.1	86.5:1 ± 1.7	33.4 ± 0.4	7.4 ± 0.2	1.0 ± 0.03	173 ± 9	25727 ± 1737	7	4	2
29/11	17.2:1 ± 0.9	6.7:1 ± 0.2	115:1 ± 3.8	39.9 ± 0.5	7.0 ± 0.2	0.9 ± 0.05	177 ± 8	29985 ± 4063	6	2	2
13/12	19.9:1 ± 0.7	6.0:1 ± 0.1	122:1 ± 2.2	37.5 ± 0.2	7.2 ± 0.2	0.8 ± NA	186 ± 6	26090 ± 2725	9	7	2
15/12	18.9:1 ± 1.2	6.2:1 ± 0.1	118:1 ± 6.7	41.2 ± 0.6	7.7 ± 0.1	0.9 ± 0.06	193 ± 12	27307 ± 3001	6	2	2

Reviewer #2
- Please, plot the filtration rate and ingestion against cell volume and food concentration.
The grazing rates should not vary this much with no reason.

Response These figures are shown above (Figure R2), and will be supplied as Figure S1 in Supplementary Material. The grazing rates are related to food concentration, however this variation is as expected when compared with previous published experiments (reviewed in Paffenhofer, 1994) and this change in ingestion did not have an effect on R or EPR due to the availability of saturating concentrations of food carbon. See discussion above.

Reviewer #2
- There is something wrong in the copepod size (row 245), T. longicornis females are around 1000 µm.

Response The female prosome length varied from 650 µm to 900 µm. The results section 3.2 has been corrected.

Reviewer #2
- Rows 268-269: According your grazing data T. longicornis was feeding in these experiments.

Response Agreed, the text was ambiguous. R_f and R_u of *T.longicornis* are not significantly different at low food qualities, so although *R. salina* are ingested by *T. longicornis*, since there is no increase of R with feeding, we assume that *R. salina* is not metabolically processed. The Results section 3.2 has been amended.

Reviewer #2
- 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.

Response Figure R5 showing respiration and egg production as functions of ingestion in terms of C, N and P will be placed in Supplementary Material as Figure S3.

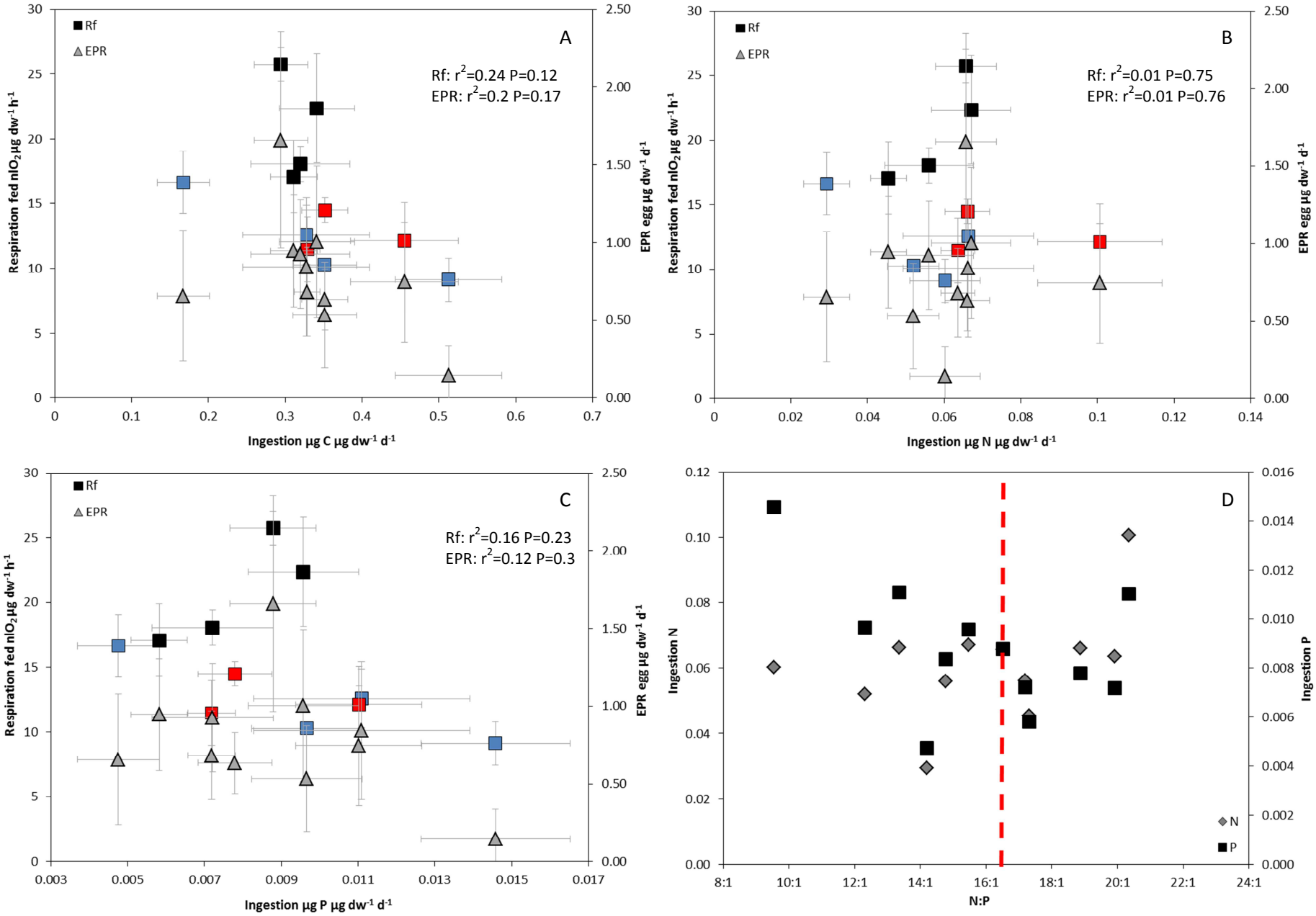
A
Rf
EPR
Rf: r2=0.24 P=0.12
EPR: r2=0.2 P=0.17
Respiration fed nlO2 µg dw-1 h-1
EPR egg µg dw-1 d-1
Ingestion µg C µg dw-1 d-1
B
Rf: r2=0.01 P=0.75
EPR: r2=0.01 P=0.76
Ingestion µg N µg dw-1 d-1
C
Rf: r2=0.16 P=0.23
EPR: r2=0.12 P=0.3
Ingestion µg P µg dw-1 d-1
D
Ingestion N
Ingestion P
N:P
N
P

Figure R5: Weight specific respiration and EPR as a function of A) carbon ingestion, B) nitrogen ingestion, and C) phosphorus ingestion. The respiration data are coloured according to balanced (black, 15.5:1>N:P<17.5:1), limited (blue, N:P 12-15 and 17-20) and extremely limited (red, N:P <12:1 and N:P>20:1) N:P ratios. D) N and P ingestion rates as a function of *R. salina* N:P ratio. Data from 11, 20 and 23 July are not shown.

The small number of data points (average n=5 each section) makes statistical analysis problematic. However for *R. salina* N:P ratios less than 16, the relationship between P ingestion and N:P ratio has a negative trend as the optimum N:P is approached (r^2=0.45 p=0.1) and for N:P ratios greater than 16, the relationship between N ingestion and N:P ratio has a positive though not significant trend as the optimum ratio is exceeded (r^2=0.45 p=0.1).

This corresponds with data showing that N ingestion of *Acartia tonsa* was significantly higher for nitrogen replete than nitrogen depleted *Thalassiosira weissflogii* cells (Kiorboe, 1989).

In addition, we would like to thank Reviewer #2 for this question which prompted us to review the conversion of ingestion into C and N units. Rather than the 0.03 µg C egg^{-1} (Kiorboe et al. 1985) that we used in the submitted manuscript, we have re-calculated ingestion in the revised manuscript using 0.0833 µg C egg^{-1} (Dam and Lopes, 2003). We have amended the Methods section 2.6 and data in Table 3. We've also added to the text the calculation of ingestion in N and P units using data from Table 1 for the figures in Supplementary Material.

As can be seen from Figure R6, GGE in units of both carbon and nitrogen peak at an N:P ratio of 16. This was mentioned in the results section of the submitted manuscript. We have now made this more explicit in the text (in Results section 3.3), and will include Figure R6 in Supplementary Material as Figure S4. As far as we are aware, there isn't a published conversion between egg size and P content, and therefore we cannot calculate either EPR or GGE in units of P. Nevertheless, we believe Figures R5 and R6 show that the ingestion of nitrogen and phosphorus and the GGE of carbon and nitrogen change either side of this optimal N:P ratio.

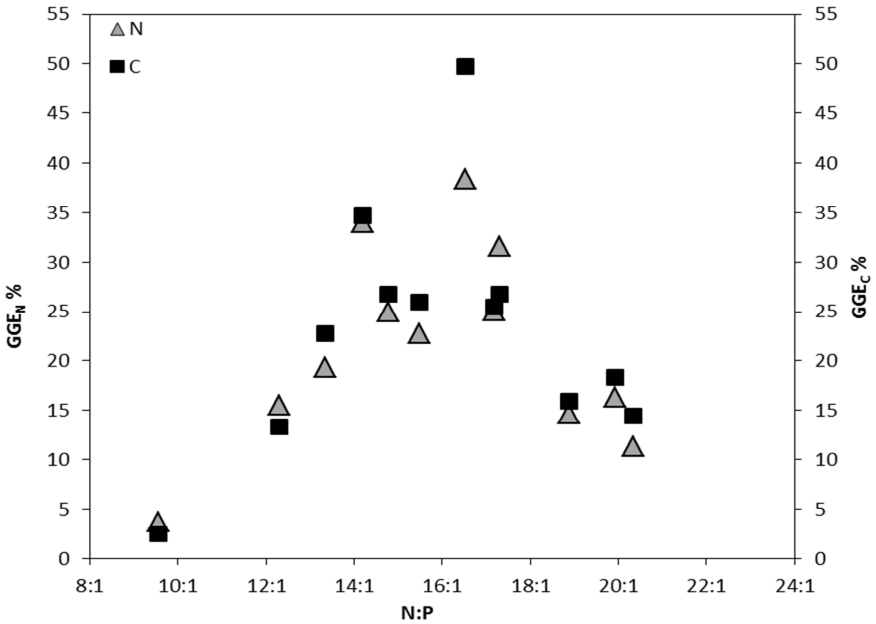

Figure R6: GGE (not including calculated data from 11, 20 and 23 July) in terms of carbon and nitrogen as a function of *R. salina* N:P ratio.

Reviewer #2
- Rows 369-370: Please compare your results (the rates, ratios etc.) to earlier studies, e.g., those of Kiørboe or Checkley.

Response We have reviewed the papers of Checkley (1980) and Kiorboe (1989) and compared our results to theirs where appropriate. Checkley (1980) compares GGE in C and N units with C:N ratio, but only for data where ingestion is below the critical value and linearly related to egg production rate. Kiorboe (1989) shows the relationship between algal nitrogen concentration and N ingestion of *Acartia tonsa*.

Reviewer #2
- Discussion, chapter 4.3 onwards. For reasons listed above, I would leave all this out, and concentrate the manuscript on the experimental results.

Response We disagree with the Reviewer and, for reasons given above, prefer to leave this section in, albeit written more succinctly. The experimental results show for the first time a dramatic decline in metabolism when algal food is P limited, and the calculated carbon budgets suggest a shift from resource allocation to reproduction, to resource allocation to maintenance, ultimately causing a species-specific decline in gross growth efficiency which can be expected to result in a decline in biomass of the population. It would be remiss of us not to at least begin to search for field data which supports or refutes this finding. We have reorganised and shortened the Ecological Implications section of the revised manuscript.

In responding to reviewers' comments and comparing our data to literature values we found an error in our calculation of respiration – this has now been corrected in the appropriate figures (1, 2 and 3) and tables (2, 3 and 4).

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