Biogeosciences
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Discussions

Interactive comment on "High temperature decreases the PIC / POC ratio and increases phosphorus requirements in *Coccolithus pelagicus* (Haptophyta)" *by* A. C. Gerecht et al.

A. C. Gerecht et al.

a.c.gerecht@ibv.uio.no

Received and published: 10 April 2014

We would like to thank Anonymous referee #1 for the comments on the manuscript and will address the specific comments below.

P 1022 L 12: is braarudii a species or a sub-species?

Sáez et al. (2003) observed levels of DNA variation between the morphotypes "braarudii" and "pelagicus" that support their reproductive isolation and therefore their separation at species level. Although these authors raise the "braarudii" morphotype to species level in their taxonomical considerations, they do not carry out a formal description and "algaebase.org" still lists "Coccolithus pelagicus subsp. braarudii (Gaarder)

Geisen et al. 2002" as a subspecies. Although both alternatives are considered valid (Jordan et al. 2005), we prefer the current taxonomically accepted names Coccolithus pelagicus subsp. pelagicus and C. pelagicus subsp. braarudii. To clarify this we have modified P 1024 L 15-18 of the original BGD submission to read:

"Coccolithus pelagicus is divided into at least two morphotypes on the basis of hetero-coccolith size and distribution (Geisen et al., 2002; Sáez et al., 2003). Although genetic infomation suggests that they are separated at the species level (Saez et al., 2003), we here use the division of the two morphotypes into subspecies, Coccolithus pelagicus subsp. braarudii (Gaarder) Geisen et al., 2002 and C. pelagicus subsp. pelagicus, according to Geisen et al. (2002) as the genetic and morphological differentiations are slight (Jordan et al., 2005)."

P 1022 L 16: please give the temperatures rather than just the difference between them.

The abstract has been changed to read as follows:

"One of the subspecies was subjected to a 5°C temperature increase from 10°C to 15°C, which did not affect exponential growth rates either, but nearly doubled cellular P-content under both high and low phosphate availability."

P 1022 L 18-19: this is not in accordance with the data. In pelagicus 15C, P-limitation does affect PIC/POC.

The statement "The PIC /POC ratio did not differ between P-limited and nutrient-replete cultures" only refers to the response of the strains under "normal" temperature (15°C for subsp. braarudii and 10°C for subsp. pelagicus). The text has been changed to specify this:

"This temperature increase reduced the PIC/POC-ratio by 40–60%, whereas the PIC/POC-ratio did not differ between P-limited and nutrient-replete cultures when the subspecies were grown near their respective isolation temperature."

P 1025 L 8: Ian Probert is an author. So I don't think that a recommendation by him needs to be mentioned.

This has been removed and the text now reads:

"Clonal batch cultures were grown in triplicate in sterile-filtered modified K/2 medium (Keller et al., 1987; with the following modifications: omission of Tris and Si, addition of NiCl2·6H2O (3.14 nM), and increase of EDTA-concentrations (5.85 μM)) at two initial phosphate concentrations."

P 1025 L 19: How was the acclimation done in case of P-limitation? The latter is in batch mode a transient stage, so it is difficult to imagine how one can acclimatise a culture to a non-constant condition.

Acclimation cultures were kept in the respective growth medium (1 and 10 μM phosphate) in the climate chambers under the same light and temperature conditions as the following experiment. The cultures were therefore acclimated to the experimental light and temperature conditions whereas P-limitation was induced during the course of the experiment. The Materials & Methods section has been modified accordingly:

"Cells were acclimated to experimental light and temperature conditions for at least 10 generations before starting the experiment."

P 1025 L 22: the light intensity probably means light limitation. Why was that chosen?

Although other studies e.g. Langer et al. (2006) used higher light intensities (350 μmol photons m-2 s-1), reaching higher exponential growth rates (up to 0.78 d-1), the chosen light intensity ($\sim$100 μmol photons m-2 s-1) was judged sufficient for exponential growth and was similar to that used in other studies, e.g. Taylor et al. (2007). These authors used the same light intensity as us and although growth rates were not reported, they found coccolith production to be saturated at this light level.

P 1026 L 4: growth rate is calculated by exponential regression of the cell density vs time curve, not linear regression.

C949

This should read:

"Exponential growth rates (μmax) were calculated by linear regression of log-transformed cell densities in exponential phase over time."

P 1032 L 5: The PIC/POC response in Ehux is strain-specific, and probably method-specific.

This part of the text has been moved to the introduction as it is considered more relevant for the motivation behind this study than for the interpretation of the obtained results. It has also been modified to include the variability of the effect of macronutrient limitation on the PIC/POC-ratio of E. huxleyi as described in the literature, including the more recent publications of Borchard et al. (2011) and Langer et al. (2013).

However, we do cite Riegman et al. (2000) and Paasche (1998) as two case studies where the PIC/POC-ratio increases with decreasing nutrient availability because of a decrease in POC production rate. We have calculated production rates from the growth rates and POC and PIC quota reported in Table 1 of the original publication of Riegman et al. (2000) (see Supplement to this comment). These authors clearly show that whereas POC production rates decrease with decreasing P-availability, PIC production rates are unaffected, leading to an increase in the PIC/POC-ratio with decreasing P-availability (we do not consider the decrease in PIC production from 1.3 $\pm$ 0.3 to 1.1 $\pm$ 0.2 pg cell-1 d-1 from the second lowest (0.29 d-1) to the lowest (0.14 d-1) dilution rate to be a significant signal because all other dilution rates resulted in PIC production rates of ca. 1.3 $\pm$ 0.5).

We also tried to extract the data from Figures 3 and 5 of Paasche (1998) to calculate production rates. Although this interpretation should be treated with caution as we did not have the original data, it would seem that also in this study, PIC production is unaffected by the decrease in dilution rate from 0.76 to 0.46 d-1 in P-limited chemostats, whereas POC production decreases towards the lower dilution rate (0.46 d-1). This led to an increase in the PIC/POC-ratio at this lower dilution rate (see Supplement to this

C950

comment).

P 1033 L 12: statement not correct. In pelagicus 15C, P-limitation caused PIC/POC to rise by almost a factor of 2.

This has been changed to read:

"Whereas P-limitation did not affect the PIC/POC-ratio when the subspecies were grown near their respective isolation temperature, elevated temperature decreased the PIC/POC-ratio by more than half in high-P cultures of subsp. pelagicus."

P 1033 L 14: not correct. In fact PIC/POC is below unity in pelagicus 15C only.

This refers only to subsp. pelagicus grown at 15°C because the same temperature is considered "normal" (i.e. near the original isolation temperature) for subsp. braarudii. The statement has been modified to specify this and to incorporate the suggestion of J.-P. Gattuso (comment to this manuscript, SC C6) as to the incorrect threshold of "1" in interpreting PIC/POC-ratios. The text now reads:

"PIC/POC-ratios were below one in both high-P and low-P treatments of subsp. pelagicus grown at high temperature (15°C)."

P 1033 L 16: not correct. POC quotas decreased in both high and low P.

In comparing subsp. pelagicus cultures grown at 15°C and 10°C, we have considered only the main experiments as we do not have information on PIC values for the additional experiments (see Table 2b; and previous reply to referee #1: C60-C63). Comparing the two temperatures (10°C vs. 15°C) for the main experiments (Table R2a), it is clear that POC quotas were not significantly reduced in either high-P or low-P cultures (t-test, p >0.05).

P 1033 L 17: not correct: In high P, elevated temperature caused POC production to decrease by 22%.

Considering only the POC production of the main experiments (see above comment),

C951

POC production actually increased by ca. 20% in high-P cultures of subsp. pelagicus at 15°C compared to 10°C. The statement has therefore been changed to read:

"In high-P cultures, POC production increased by ca. 20% at elevated temperature whereas PIC production declined by ca. 60%."

P 1033 L 18: PIC production declined by 58%. So that's ca. 60%, rather than ca. 50%

This has been changed to read ca. 60% as suggested.

P 1034 L 5: temperature-stress treatment is supposed to mean pelagicus 15C, I guess.

That is correct. The text has been changed to read:

"Within the high temperate treatment (15°C) of subsp. pelagicus, the PIC/POC-ratio, although still lower than at 10°C, was significantly higher under P-limitation due to a higher PIC quota."

P 1034 L 5-19: the discussion is not convincing, because if P limitation affects primarily POC production, the PIC/POC ratio should always increase, which it clearly does not (not even in the dataset presented here, let alone in other datasets in the literature).

This part of the discussion has been modified to more clearly outline the reasoning behind this hypothesis (see reply to comment P 1034 L 25 - P 1035 L 12 further down).

P 1034 L 8: which offset?

The text has been changed to specify the offset:

"However, the difference in PIC quota between high-P and low-P cultures at this temperature may be due to these treatments being sampled in different growth phases."

P 1034 L 18: what does "calcification" mean? The correct term is PIC quota.

"Calcification" in this statement refers to PIC production, not quota. However, this part of the text has been modified and no longer includes this statement.

C952

Also the statement about the Riegman paper is not correct. In the latter PIC quota increased by a factor of ca 4, and POC quota increased by a factor of ca 2.

In quoting the Riegman paper we refer to the production rates, not the quota as the continuous set-up used by Riegman et al. (2000) allows a comparison between production rates (due to constant growth rates). We have calculated production rates from the growth rates and POC and PIC quota reported in the original publication (see Supplement to this comment and reply to comment P 1032 L 5 above).

P 1034 L 22: The hypothesis in the cited paper was tested in Langer et al. 2013 (JEMBE 443, 155-161). Please cite also the latter paper here.

This part of the text has been modified and no longer includes the cited hypothesis (inapplicability of calculating production rates in batch culture). However, the citation has been added to the parts of the text that do mention this hypothesis (e.g. section 2.3.2 of the Materials & Methods).

P 1034 L 23: not correct. PIC/POC increases in pelagicus 15C in response to P-limitation.

This part of the text has been modified and no longer includes this statement.

The discussion P 1034 L 25 - P 1035 L 12 is completely beside the point. For pelagicus it is obvious, but not easily explained, that temperature changes the PIC/POC response to P limitation.

We have modified the relevant parts of the discussion to better illustrate the proposed hypothesis for explaining why temperature changes the PIC/POC-response to P-limitation. First of all, we agree that the reason for the decrease in PIC quota at high temperature is unclear. However, we have shown that at 15°C the coccolith coverage of subsp. pelagicus cells is reduced.

The increase in PIC quota from high-P to low-P cultures of subsp. pelagicus at this temperature (15°C) can therefore be due to POC production being more dependent

on P-availability than PIC production. We will outline this hypothesis in detail in the following.

PIC production cannot increase due to P-limitation at "normal" temperature when the cell is fully covered by a single layer of coccoliths. Coccolithus pelagicus has not been described to overproduce coccoliths as occurs in E. huxleyi. Therefore, there are spatial constraints to coccolith production i.e. once the cell is covered by a complete single layer of coccoliths, coccolith (and thereby PIC) production stops as long as the cells have stopped dividing (which is the case in low-P cultures which have reached stationary phase due to P-limitation).

This spatial constraint is alleviated at high temperature in subsp. pelagicus (15°C) due to reduced coccolith coverage. In high-P cultures harvested in exponential phase cell division rates (and thereby POC production) are relatively constant. However, in low-P cultures, going into stationary phase, cell division rate (and thereby POC production) decreases. Assuming that PIC production is less constrained by P-availability than POC production, reduced POC production in low-P cultures can allow PIC (coccolith) production to "catch up" as there is space on the cell for placing further coccoliths. This leads to an increase in the PIC/POC-ratio. Some authors (e.g. Fritz et al. 1999, Borchard et al., 2011) observed a decline in PIC production once POC production decreased due to nutrient limitation, leading to stable PIC/POC-ratios. However, other authors have observed that PIC production can continue once POC production declines (e.g. Riegman et al, 2000; Paasche, 1998). We agree that Riegman et al. (2000) make a convincing case to show that PIC production can be sustained during POC production decline in P-limited chemostats at least in some strains of E. huxleyi (see also Supplement to this comment). Also the data of Paasche & Brubak (1994) and Paasche (1998) highlight the same trend. We therefore suggest that in some cases, POC production can be more constrained than PIC production by the availability of nutrients. This could apply especially to P, which is fundamental for cell division. A stronger constraint on POC than PIC production would explain the increase in PIC

quota in low-P cultures going into stationary phase compared to high-P cultures harvested in exponential phase. Again, this increase is only observed at high temperature (15°C) in subsp. pelagicus in which coccolith coverage is anyway reduced. There is therefore space for the production of additional coccoliths. The same increase is not observed at "normal" temperature (subsp. braarudii 15°C, subsp. pelagicus 10°C) because of the above mentioned spatial constraints to coccolith production.

Could it be that pelagicus suffers from heat damage at 15C? The higher percentage of malformations at 15C points indeed to heat damage (see also Langer et al. 2010 J. Phycol. 46, 1252–1256).

Subsp. pelagicus responds to increased temperature (from 10°C to 15°C) by exhibiting reduced PIC production, increased P-requirements and increased coccolith malformations. All these physiological effects point towards "heat damage" i.e. a detrimental effect of high temperature in this subarctic subspecies. This is analogous to the negative effect of increased temperature on coccolith morphology described by Langer et al. (2010) for E. huxleyi. This reference has therefore been added as a reference for increased malformation of coccoliths at high temperature.

Why would higher than optimum temperature increase P requirement?

That is indeed a fundamental question, the answer of which may tentatively relate to enhanced RNA production. A sentence about the possibility of increased P in high temperature cultures due to increased RNA content has been added:

"As most of cellular P is to be found in RNA (Geider and La Roche, 2002), the higher POP content of subsp. pelagicus grown at high temperature (15°C) could relate to increased RNA content due to the expression of genes related to temperature stress responses. However, the nitrogen content of the cells did not increase, making an increase in enzyme production an unlikely explanation."

P 1036 L 11: Culture artefacts can, at present, not be explained entirely satisfactorily,

but there has been some progress since 2009. It was shown that high cell densities and a lack of mixing lead to higher percentages of malformations in Ehux (Langer et al. 2013 Helgoland Marine Research 67, 359-369).

A short discussion of factors influencing coccolith malformation, such as high cell densities and lack of mixing, has been added to this part of the discussion.

"Malformations in control cultures may arise from high cell densities (Langer et al., 2013b) which may explain the lower level of malformations observed by Rickaby et al. (2010) in highly dilute batch cultures (<2.300 cells mL-1) compared to our higher cell concentrations (ca. 13.000 cells mL-1). However, the 50% malformed coccoliths reported by Langer et al. (2006) were observed in batch cultures at cell concentrations of ca. 6.000 cells mL-1, indicating that the percentage of malformations may not be constant over time (Langer et al., 2013b). Another factor leading to malformations in culture is a lack of mixing (Langer et al., 2013b). Our cultures were agitated only once a day which may have contributed to the high degree of malformation observed in our control cultures."

P 1036 L 14-17: This comparison nicely illustrates another fact about culture artefacts, i.e. that they are not constant over time.

This aspect has also been added to the discussion mentioned above (comment P 1036 L 11).

P 1036 L 17-19: The statement about Ehux is not correct. Neither of these studies show that Ehux increases the percentage of malformations in response to P limitation (see also discussion in Langer et al. 2012 JEMBE 413, 131-137).

Langer et al. (2012) discuss that an increase in malformations <10% should be interpreted with caution. We agree with the referee that the reported increases in malformation in the two citations (Båtvik et al., 1997; Paasche, 1998) are slight. The text has therefore been changed to read as follows:

"Nutrient limitation has previously been suggested to increase malformations in E. hux-leyi in both mesocosm and laboratory experiments (e.g. Båtvik et al., 1997; Paasche, 1998). However, the malformation increases described by these authors are slight and/or poorly quantified and Langer et al. (2012) argued that a difference of less than 10% in malformations may be within the range of natural variation. Also, Langer et al. (2012) failed to observe an effect of nutrient availability on coccolith morphology in C. leptoporus and concluded that the influence of nutrient availability on the coccolith machinery is negligible."

To sum up, the present study is the first one to report macro-nutrient limitation induced malformations.

This has been highlighted.

"This study is therefore the first to show that coccolith morphology can be significantly (>10%) modified by limitation by a macronutrient, in this case phosphate."

P 1036 L 22: by "outside" the authors probably mean "above the optimum".

This is correct. The text has been changed as follows:

"This temperature was probably above the temperature tolerance for this subarctic strain and increased malformations could have been related to a stress response to high temperature."

P 1037 L 7: increased PIC/POC is only true for some Ehux strains (see above).

This part has been removed.

P 1037 L 9: there might in principle be an upper constraint but this is definitely not the point here (see above for details).

See reply to the above comment P 1034 L 25 - P 1035 L 12.

P 1037 L 12: not true for pelagicus at low P, if one believes the table, precisely the

quota data in the table and not the calculated PIC/POC, because the latter does not tally with the quota data.

The two experiments (main and additional) have now been separated and only the main experiments used to examine differences in the PIC/POC-ratio among the treatments as PIC quotas are not available for the additional experiment.

P 1037 L 20: in Ehux there are even strain-specific differences (see above).

The text has been modified to read:

"Species- and strain-specific differences in physiology need to be taken into considera-tion when predicting general responses of coccolithophores to changing environmental parameters and possible changes in CO2-feedback to the atmosphere."

Table 1 pelagicus T0 low P 15C: the CO2 numbers are swapped I guess.

This has been fixed.

Please put all data on the cells in the table, including loose and attached coccoliths and morphology. Especially the data on morphology need to be in a table, because the figure does not contain standard deviation.

We do not have information on loose vs. attached coccoliths as the total coccolith counts were carried out on dispersed samples.

A new Table 3 will be included containing morphological data such as coccosphere diameter and coccolith coverage, coccolith length and thickness (POL), and coccol-ith malformations. Figure 5 (coccolith morphology) has now been split into the two separate experiments (main and additional) with the triplicate batch cultures presented separately to give an idea of the degree of variation among replicate cultures. In the new Table 3, a 95% confidence interval is given for malformations because percentage data is not normally distributed and a standard deviation is therefore not applicable.

Figure 1: how is n=6 possible with triplicate incubations?

The experiments were carried out in duplicate (main and additional experiments) therefore the sample number is 2 experimental runs * 3 replicate batch cultures = 6 incubations. However, these experiments are now presented separately (see Tables 1, 2, 3).

References:

Gattuso, J.-P., Biogeosciences Discuss., 11, C6, 2014.

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Riegman, R., Stolte, W., Noordeloos, A. A. M., and Slezak, D.: Nutrient uptake and alkaline phosphatase (EC 3:1:3:1) activity of Emiliania huxleyi (Prymnesiophyceae) during growth under N and P limitation in continuous cultures, J. Phycol., 36, 87-96, 2000.

Taylor, A., Russel, M. A., Harper, G. M., Collins, T., and Brownlee, C.: Dynamics of formation and secretion of heterococcoliths by Coccolithus pelagicus ssp. braarudii, Eur. J. Phycol., 42, 125-136, 2007.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/11/C947/2014/bgd-11-C947-2014-supplement.pdf

Interactive comment on Biogeosciences Discuss., 11, 1021, 2014.