Rreferee #1

While the authors have successfully addressed many of my specific comments, they have not addressed the global issue of the manuscript being vague and unspecific to the point where it is not clear that the data matches the conclusions the authors are making. It's very possible they do match, but the way the manuscript is written, it is not currently clear. The manuscript still suffers from major flaws that prevent it to be published in its current form. Most concerning of which is the lack of specificity that the authors use in their language. It is impossible for the reader to know exactly what results the authors are referring to.

For example: throughout the manuscript the authors use phytoplankton biomass and chlorophyll concentration interchangeably (e.g., ln 321, 355). The authors measured chlorophyll concentration, not biomass. It is strongly established that chlorophyll concentration to cellular carbon (biomass) ratios are highly variable in phytoplankton, especially across seasonal changes in light and nutrient concentration (which is the context for the authors experiments). It is not valid to say that phytoplankton biomass is being estimated by chlorophyll concentration. I do think that measuring chlorophyll concentration is a valid method of tracking phytoplankton, but the authors need to be specific throughout the manuscript about what they are actually measuring, and make sure that the conclusions they are making can be supported by their actual data. Reporting changes in chlorophyll concentration have a dramatically different physiological and ecological implications than reporting changes in biomass. I can't be sure what they mean with the manuscript in its current form.

In sum, this manuscript has a lot of potential. I was (and am) excited to see this experiment, and I do think the data should eventually be published. However, as it stands currently, the authors have not done their due diligence making sure that the manuscript is clear, specific, and ready for publication.

Referee #2

Review of ”Spatial and temporal variability in the response of phytoplankton and bacterioplankton to B-vitamin amendments in an upwelling system” by Joglar et al.

General comments

This manuscript covers a very interesting and highly relevant topic, which the authors focuses on in the manuscript. Dynamics of B-vitamins in the worlds ocean is not often studied and this manuscript attempts to provide important information on this topic. The sampling campaign is definitely impressive, as well as the work that went in to the study. I don’t agree with all the comments from the previous reviewers, for instance I find the use of response ratios very informative and a great display of the results. With that said, I have some comments and concerns with the manuscript that need to be addressed.

One main problem with the manuscript is that only one of the vitamins investigated is analyzed. I realize this may be due to problem quantifying B1 in natural sea water, which the authors can state more clearly. It would also have been very interesting to have quantified the cellular content in the two size fractions, but unfortunately this was not done.

Generally, is there any benefit of using st3 and st6 instead of coastal and oceanic station? I feel the readability would increase if you used coastal and oceanic instead.

When referring to figures, state which of the figures, a, b or c. you refer to. Also, look over all figures so that all are labelled a, b, c …. For fig. 2 and 3 I’m having troubles seeing the benefits of having several a’s, several b’s etc. I would like to see labelling a-r instead for fig. 2, then you can refer to the specific mosaic.

I would like to see a more accurate reporting of statistics. Please provide statistics value (t, F, df) when appropriate.

In my opinion, the results should be presented as averages, per station and cruise and ignore 3a, 3b, 3c etc. I understand that a tremendous amount of work has gone into this experiment, but I believe that the paper would benefit from a more succinct and concise result section.

Specific comments

Abstract

L15; "… unimportant, …" – I would suggest changing wording, as you cannot know if it is unimportant or not. Maybe "slight" or "limited"?

L15; how can an "unimportant" variability lead to the assumption that there are factors operating at other scales? Requires clarification.

L20; change "alone" to solely?

L22-24; auxotrophy is also high in phytoplankton, causing the argument to halter a bit. I would suggest mentioning this as well and combine it with bacteria dependence.

Introduction

L34; state which toxic episodes you refer to.

L60-61; I would suggest reading Cruz-Lopez et al. 2016.

L69-74; I would suggest reviewing if you really need all references to say what you want to say. In a relatively short sentence, you use 13 references.

L100 & L110; decide if you use numbers or text, 36 or thirty-six, and use throughout.

L105; change "synthetize" to synthesize.

Methods

L119; What is the timeframe between a, b and c? Looking at fig 1 I realized I can figure it out, but it is a very unclear way to present samplings.

L120-123; To increase clarity, I would recommend to state that surface is 5m deep more clearly.

L123; State which occasion this sampling failed.

L128-129; do you refer to the t0 for each experiment (a, b and c)? Needs clarification.

L129-132; Does the UI provide you with important information?, now sentence feels a bit dropped in the text.

L133-134; What about small zooplankton, copepodites and nauplii? Did you check for this, if so it should be stated. If not, the potential impact of these should be taken into account for.

L138-144; This is a very confusing way to present the treatments. I would suggest providing all of this important information in a table instead. Additionally, the rationale behind the levels of nutrients and vitamins should be given.

L147-150; This is unclear to read. First it is natural conditions, then the conditions were reproduced? How was this done? What screens are you referring to?

L152; change to "t0"?

L160-162; Revise sentence. Suggestion "Samples were incubated 20 min for the fixative to act on cells, immersed in liquid nitrogen for 15 min before being frozen at -80ºC."

L169-170; Could the usage of two different factors cause a problem in the interpretation of the data, when comparing coast and oceanic station?

L173; "… first place…" before all other variables? If so, please clarify.

L174-177; Revise sentence. Suggestion "Polyethylene bottles (50 ml, pre cleaned with 5% HCl were filled with the sample using contamination-free plastic gloves and immediately frozen at –20°C until analysis, using standard colorimetric methods with a Bran-Luebbe segmented flow analyzer (Hansen and Grasshoff 1983)." Or did I misunderstood "free-contamination"?

L182; Unfortunately, you only have samples for dissolved B12. This should be specified.

L183; Specify when the fifth or sixth day was sampled, as it can influence the results.

L188; Do you refer to leftover water? If so, change wording. If not, clarify.

L199-200; State which values apply for length, inner diameter and particle size of column.

L211; You have not used subscript before, change to B12.

L211-212; State which congener had which LOD.

L212; If the case, state that 0.05 is for cyanocobalamin, CNB12. Also, change to hydroxocobalamin.

L214; You have not stated what CNB12 is.

L219; Why was plankton community sampled day 1, 2, 4, 6, while B12 was sampled day 1, 3, 5/6?

L222; Change "litters" to liters.

L237-238; Can you update with the accession numbers?

L245-247; How can this be? Is it fragments of cells going through the 3 µm filter? Would benefit from an explanation for this.

L251-252; Please provide the rationale for this procedure.

L265; "… if necessary to attain normality". Was this not always the case, do you have some samplings where the data was not normalized and some where it is? If so, you should state when this was the case and discuss how this might affect the results and conclusions drawn from them.

L266; When "standardizing", do you refer to using the corrected p value?

L267-273; Why using ANOVA and Z-test? The reasoning behind this choice should be given.

L276-281; how was this data normalized? Change to "chl-a and bacterial biomass".

L283; How many permutations were performed? Should be stated.

L285; I would suggest using bacterioplankton prior to this. Use already in introduction over bacteria.

L287; "… selection criteria)…". Remove ")".

L291; change “responses” to limitations?

Results

L294-312; This part is very descriptive, it would benefit from being shortened, to get to the more interesting findings of you paper.

L294; Here and elsewhere, when referring to figures, state which of the figures, a, b or c. you refer to. See general comment.

L296; change “meters” to m?

L310; change “an” to and.

L313-320; why not presenting DIP values by themselves, but only in DIN:DIP ratio?

L319; add 16:1 to Redfield ratio. (…Redfield ratio (16:1))

L321; change “greatly varied” to varied greatly?

L323-324; “cruise” is redundant.

L325; change “bacterial biomass” to BB, as you state this in L323.

L332; Information on MDS analysis is missing from statistics section. Please add information regarding this analysis.

L332-333; Please clarify. Suggestion “… relatively reduced variability within period”.

L338; *Mamiellophyceae* is not included in the legend in. As they are the first once you mention, I would suggest including them in the figure 4.

L342; Explain what MALV refers to.

L343; Change to “Flavobacteriales and Rhodobacteriales…”

L343; The reference to fig 4b is incorrect. See general comment regarding labelling of figure and mosaics.

L345; See comment L338. Also, which cyanobacteria are you referring to?

L346; See comment L343.

L347; See comment L338, regarding Archaea.

L349; change “Mean” to Average?

L350; Here and elsewhere, provide t value.

L351; There is no fig 4c. See general comment regarding labelling of figure and mosaics.

L354; change “evolution” to development?

L356; “… in most …” Too general. Please specify the proportion at least.

L361-365; This section does not relate to response ratios (even if stated in L361). Please rephrase.

L362-363 & 367; Here and elsewhere, provide F value and df.

L367-369; Revise English.

L369; Here and elsewhere, provide F value and df.

L369-372; Revise English.

L373-375; Maybe state in which experiments this happens? Similar to L387-390.

L373-383; I would suggest restructuring for clarity. As now it is very difficult to understand when different responses occurred.

L377-378; Maybe state in which experiments this happens? Similar to L387-390.

L391-395; This part appears to belong in Material and Methods section.

L395; 4 sites? 2 stations and 2 depths? Please clarify.

L397-400; To me, these results are the most interesting. I would suggest restructuring the result, putting emphasis on the response ratios.

L405; "Most positive…". State proportion (%).

L418-422; This part appears to belong in Material and Methods section.

L422-423; What was Spearman Rho correlation with eukaryotic community composition.

L426; Where does the 78% originate from? State each dimensions contribution.

L430-431; State each dimensions contribution to the 59.4%.

L431-433; Revise English. Also, I'm struggling to see that the stations are actually separated.

L434; "… highly and positively correlated…". Revise English.

Discussion

L443-445; As you don't have measurements on B1, this statement is not fully true. Please tone down this statement.

L446; What expectations are you referring to? These should be stated more clearly before.

L448-452. What about predation pressure? Cellular demand of B vitamins? Actual cellular content of B vitamins? Should be expanded to include more potential explanations.

L452-454; In my opinion, this should have been done for all of the results. I understand that a tremendous amount of work has gone into this experiment, but I believe that the paper would benefit from a more succinct and concise result section.

L456 "… frequent but relatively moderate…". What does this mean, please clarify.

L461-464; What results are this statement based on?

L497-500; Highly speculative. Please rephrase to tone down this statement.

L521; change "potentially" to potential

L522-546; I would suggest reading Fridolfsson et al. 2018 and 2019, as well as Sylvander et al. 2013 to provide additional depth to the discussion on B1 and B12 amendments.

L563-566; Shouldn't dinoflagellates pop out in the analysis then?

L567; Why "strikingly"?

L570; change "revel" to reveal?

L576; Which "predation" are you referring to? Please clarify.

L582-583; What about uptake rates? I would suggest reading Koch et al. 2011, 2012, 2013 and discuss.

L588; "… B12 producers and B1 consumers." This is extremely generalized and implies that you can determine this in your paper. This is not fully true, especially for B1 as you don't have measurement for this B vitamin.

L590; "… cope with B vitamin shortage…". See L588. Once again, it is unfortunate, but you don't have measurements for B1 so your conclusions regarding this B vitamin should be toned down.

Figure captions

Please make sure that everything in your graphs can be identified. E.g fig 1, that cruises is illustrated by lines (in 1c legend), dots in fig 2, what 16:1 line refer to in fig 3.

Also, Generally, is there any benefit of using st3 and st6 instead of coastal and oceanic station. I feel the readability would increase if you used coastal and oceanic instead.

L937-941; Change "µmol l^{-1}" to µM? Pinpoint that axes are broken. Specify what SCM means.

L942-945; If so, state that it refers to t0. Also, what are the error bars showing?

L946-949; Suggestion, …(estimated as Chl-a concentration (µg l^{-1})). Change "time-zero" to t0. Change "final-time" to endpoint. Pinpoint that axes are broken. Also, what are the error bars showing?

L950-952; Change "time-zero" to t0. Change "final-time" to endpoint. Pinpoint that axes are broken. Also, what are the error bars showing?

L953-960; I would suggest using more mosaics, a-d.

L961-968; change "… microbial plankton…" to microbial bakterioplankton, as it is only prokaryotes? Should be stated in the beginning and not at the end of the figure caption

Figures

Figure 1; Generally, is there any benefit of using st3 and st6 instead of coastal and oceanic station. I feel the readability would increase if you used coastal and oceanic instead.

Figure 2; When referring to figures, state which of the figures, a, b or c. you refer to. Also, look over all figures so that all are labelled a, b, c …. For fig. 2 and 3 I'm having troubles seeing the benefits of having several a, several b etc. I would like to see labelling a-r instead for fig. 2, then you can refer to the specific mosaic.

Figure 3; See comment for fig 2. For fig. 2 and 3 I'm having troubles seeing the benefits of having several a, several b etc. For the legend, the depth is stated as 0m and SCM, change to

"surface (5m) and SCM", as you did not sample 0m, correct? Also, state what the 16:1 line refers to. Also, I would suggest providing an average per station and cruise, and not all 3a, 3b and 3c etc, see general comments.

Figure 4; change mosaics to cover a-c, as stated in the main text. On the x-axes, the depth is stated as 0m and SCM, change to "surface (5m) and SCM", as you did not sample 0m, correct? You do not use a consistent taxonomy level, some are species whilst other groups are a combination. Could this affect your results? If not, I would still reconsider the different taxonomical levels presented.

Figure 5 and Figure 6; The colors are very difficult to distinguish. Also, I would suggest providing an average per station and cruise, and not all 3a, 3b and 3c etc, see general comments.

Figure 7; I would suggest changing the layout, to something used frequently when presenting fold change. You don't need to show 0, as every finding is around 1. See oversimplified suggestion below.

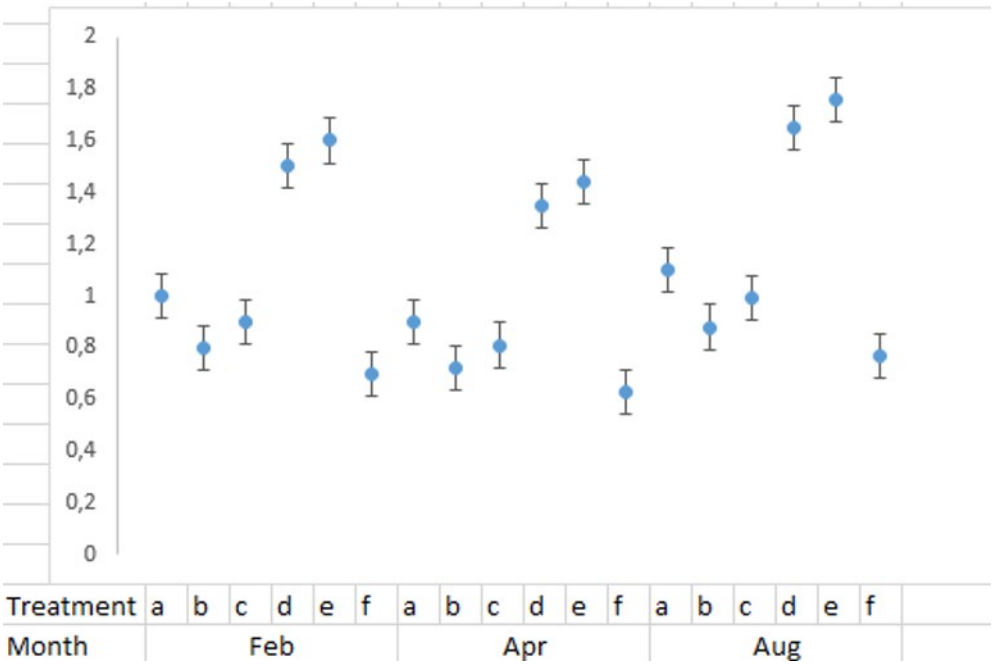

Figure 8; You do not use a consistent taxonomy level, some are species whilst other groups are a combination. Could this affect your results? If not, I would still reconsider the different taxonomical levels presented. The legend needs formatting prior to publication, much too large as it is now. The depth is stated as 0m and SCM, change to "surface (5m) and SCM", as you did not sample 0m, correct?

Supplement information

Table S2; This information is the same as in fig 3, correct? To me, this is redundant. If to be included, abbreviations in column names should be explained.

L18-27; "… experiments by the averaged…". Add divided? Change "that means" to which implies. Pinpoint that axes are broken

Figure S1; Shouldn't axes present statistics?, Percentages? The legend needs formatting prior to publication, much too large as it is now.

Figure S2; I propose including this graph over Fig 5 and 6. If included, it must be formatted to conform to the palette the authors have used, for clarity. How was these stats performed, as RR already considers the control. Clarify.

Figure S3; I would suggest changing the layout, to something used frequently when presenting fold change. You don't need to show 0, as every finding is around 1. See comment for figure 7. As it is now, it is impossible to get any valuable information from the figure.

References

Cruz-Lopez R, Maske H. (2016). The Vitamin B_1 and B_{12} Required by the Marine Dinoflagellate *Lingulodinium polyedrum* Can be Provided by its Associated Bacterial Community in Culture. Front Microbiol. 7:560. doi: 10.3389/fmicb.2016.00560.

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Koch F, Hattenrath-Lehmann TK, Goleski JA, Sañudo-Wilhelmy S, Fisher NS, Gobler CJ. (2012). Vitamin B_1 and B_{12} uptake and cycling by plankton communities in coastal ecosystems. Front Microbiol. 3:363. doi: 10.3389/fmicb.2012.00363.

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Sylvander P, Häubner N, Snoeijs P. (2013). The thiamine content of phytoplankton cells is affected by abiotic stress and growth rate. Microb Ecol. 65(3):566-77. doi: 10.1007/s00248-012-0156-1.