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Interactive comment on "Interactive effects of vertical mixing, nutrients and ultraviolet radiation: in situ photosynthetic responses of phytoplankton from high mountain lakes of Southern Europe" by E. W. Helbling et al.

P. Neale (Referee)

nealep@si.edu

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General Comments

Although there have been several studies of how vertical mixing in lakes and the ocean interacts with UVR inhibition of phytoplankton photosynthesis, this is the first study that has included a third factor, nutrient enrichment, into experiments examining the interaction. All three factors, UV/light climate, vertical mixing and nutrient inputs are expected to vary with climate change. The authors' study systems are three Spanish lakes that

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can receive nutrient pulses, for example, through Saharan dust events. They use an elegant experimental design that has a full factorial set of treatments, +/-UVR, mixed vs static, with or without nutrient enrichment. The settings of the three studied systems were considerably different, so not too surprisingly, somewhat different patterns of results were obtained for each. The authors describe a synthesis of these results into an overall interaction scheme, including data from other studies, but the support for this scheme was not so clear (so to speak). This is a novel and interesting study that is generally well presented (apart from some technical issues detailed below) but attention is needed to the interpretation of results addressing the issues that are described in the following Specific Comments section.

Specific Comments

The authors propose that lake transparency has a dominating effect on how vertical mixing modulates the impact of UVR on primary productivity. This proposition is based partly on the data presented in the paper, but also using data from other studies which actually make the most important contribution to defining a statistically significant trend in the summary figure (Fig. 7). The authors are not very specific about how these extra data points were calculated, however, the position of some points on this graph seem contrary to the conclusions made in the text of these other papers. The graph shows the UVR inhibition results from three previous studies which sampled lakes with Kd320 > 1.0 m-1 and in each case the point on the graph indicates that UVR inhibition was higher in the mixed than in the static samples, supporting the statement in the text that "In opaque lakes (i.e. with high Kd320) the inhibition was greater under mixed than under static conditions" (Pg-Lines 9805-24-25). Point #1 has a citation to Köhler et al. (2001). While Kohler et al present results for a range of different incubations, the results most relevant to this analysis appear to be model calculations for integrated production during a 4 h midday incubation of bottles that were (hypothetically) circulated (0 - 3.9 m) versus static at 1.2 m both having the same average UV exposure. The model estimated less inhibition for the mixed sample (26%) than the static sample (32%) (Köhler et al. pg. 304), so the position of this particular result in Fig. 7 (mixedstatic) would be on the negative side of the y-axis in contrast to the positive point that is shown. Point # 2 is from Hiriart-Baer and Smith, they also present model calculations which compare mixed vs static production, but in this case the opposite conclusion was reached, inhibition was greater in the mixed than in the static, mainly because of slow recovery rates This does seem to be consistent with the positive position of point # 2 on Figure 7. Point # 3 is from Villafañe et al. (2007), they measured photosynthetic efficiency (from PAM fluorescence) in fixed depth incubations vs water column samples. This study only had +/- UV treatments at the surface so it is not clear how UVR inhibition over the water column was estimated. Apparently, they interpreted differences between fixed depth and water column samples in terms of UVR exposure (though it seems PAR exposure could have been important, too). Nevertheless, they conclude that "..., vertical mixing not only counteracted the impact of UVR but also resulted in higher photosynthetic efficiency at all irradiances (Fig. 6)." The implication seems to be that inhibition was less under mixing than under static conditions, which would result in a negative point on Figure 7 in contrast to the positive point that is shown.

These three points account for most of the trend in relationship between UVR inhibition (mixed-static) and Kd in Fig. 7, so it is critical that their calculation be fully documented and any inconsistency with interpretation in the original publication be explained. Was the data specified above used to make the graph? If not please explain how the points were derived. From the way these previous results were discussed in the original papers, at least, there is not general support for the authors' contention that UV inhibition is expected to be enhanced by mixing in low nutrient, opaque lakes. Instead, mixing sometimes enhanced (study 2) and sometimes ameliorated (studies 1 and 3) inhibition in these systems. If this is the case, then neither is there a general contrast based on presence/absence of a nutrient pulse. My own sense is that multiple factors are at work here, in addition to transparency, type and extent of nutrient limitation, taxonomy, depth of mixing and temperature, all of those influencing the rates of damage and recovery which are the ultimate determinants of how UVR inhibition interacts with vertical

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mixing.

A second conclusion taken from Fig 7 is that "increased EOC values were also observed in opaque lakes under mixing conditions, and they decreased towards clear lakes" (9805-27), but this interpretation also does not seem consistent with the data presented, indeed the data seem to suggest the opposite conclusion - EOC was less under mixing. It is not stated specifically, but the implied calculation seems to be EOC(Mixed, UVR) - EOC(static, UVR), also unstated whether EOC amount or percentage was used (the latter seems more appropriate). Either way, results in Fig. 6 would not suggest a strongly positive point for LE (Kd320=2.28) shown in Fig. 7. By either measure, EOC(Mixed, UVR) is less than EOC (Static, UVR) in LE, thus the point would be expected to be negative. For LY, text (9805-2) also states "samples under mixing conditions had lower amounts of EOC than in the static ones" consistent with Fig. 6 b but Fig 7 shows a positive value for EOC(Mixed -Static). For LE, EOC was (slightly) greater in mixed than static for amount but no significant difference for percent, but a negative value is shown. Finally, a EOC (Mixed-Static) value is plotted citing Kohler et al. 2001, but they measured EOC only in mixed incubations so I don't see any way to calculate a difference in EOC between mixed vs static.

Based on these issues, it seems premature to consider the conceptual associations shown in Fig. 8 as something that applies generally to lakes, particularly the indicated directional changes in inhibition and EOC release with increased (no P) or decreased (+P) transparency.

Technical Comments:

Abstract: line 1: Wrong tense: change "had" to "has" Line 11: Correct preposition: change "associated to global change" to "associated with global change" Line 22-23 & 25: Use singular: change "Nutrients" to "Nutrient" Text (pg-line): 9793-5: Wrong tense: change "had" to "has" 9793-9: Use singular: "others" to "other" 9793-17: Redundant phrase: change " nutrients would be used-up and depleted" to "nutrients would be

depleted"

9794-16: Change "the raise of temperature" to "periods of elevated temperature" 9794-17: Awkward: instead of "traits" how about "events"? 9794-18: Missing word or typo: "from of terrigenous material"

9795-2: Verb case: change "deserve" to "deserves" 9795-3: change "pulsed" to "puls-ing"?

9797-9: Use singular: change "Nutrients" to "Nutrient"

9798-25: "The mean PAR irradiance within the epilimnion was calculated as: "Two of the lakes (LC and LY) did not have a well-defined epilimnion at the time of sampling. Clarify that what is actually being calculated is the mean PAR irradiance in the upper 3 m, the depth of operation of the circulation device. However, note that the average irradiance experienced by samples in the mixed (rotated) incubation may be somewhat different than that calculated with this formula because with a sinusoidal transport rate residence time is not equal at all depths (see discussion in Köhler et al. 2001).

9799-22: " 3 μ m Whatman GF/D filters (25 mm diameter) and then through 0.7 μ m Whatman GF/F filters (25 mm diameter)" It should be kept in mind that the minimum size of retained particles on glass fiber filters (in contrast with Nucleopore) is only nominal and depends on such factors as particle shape and filter loading.

9800-10-11: " Because the time between sampling and the saturating light pulse was in the order of a few seconds, the photochemical effective quantum yield of PSII (Y) in the light was determined (Maxwell and Johnson, 2000)." Based on the description given in 9798-13-15, a 4m, 5 mm ID, tube connecting the PAM fluorometer and the sample bag would contain a volume of 78 mL, resulting in about 19 s dark time before yield measurement at 250 mL min-1. Even a few seconds of dark are sufficient to completely reverse photochemical quenching, so it is incorrect to describe the measurement as effective photochemical quantum yield. A better description is the maximum.

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mum or intrinsic photochemical efficiency of PSII which primarily reflects the effect of non-photochemical quenching.

9804-26-27: It appears that %EOC has been calculated as the ratio EOC production to POC incorporation. The calculation of %EOC is different from other reports in the literature (c.f. Obernosterer & Herndl, 1995), it is more usual to calculate %EOC release as $100 \times EOC/(EOC+POC)$.

9807-5-8: The authors suggest that the static incubation in LE may have been light limited based on the carbon fixation results. But based on the stated light transmission data, a static incubation at 1.3 m in LE would get about 64% of surface irradiance similar to the average irradiance in the circulated incubation (63%). For the latter, Fig. 3 shows that mean irradiance was around 850 umol photons m-2 s-1 on the cloudy day and a similar mean irradiance would be expected to occur at 1.3 m. Do the authors really think that photosynthesis can be light limited at such a high irradiance? Moreover, based on the Kd320 of 2.28 m-1 (and figuring a Kd360 about half of that?), an incubation at 1.3 m is only getting around 5-10% of surface UV – a very minor supplement in photon terms.

9807-27 "however, the opposite would occur in clear lakes, provided that the irradiance conditions at the water surface are similar."

Not clear what is the contrasting result in clear lakes that leads the authors to use the word "opposite".

9809-3 typo: change "addtion" to "addition"

Fig. 4 vs Fig. 5. Considering that the values in Fig. 5 are calculated by difference from those in Fig. 4, the size of the error bars between the two figures seem inconsistent. In general, the variance of a difference (Inhibition) combines the variance of values being subtracted (UVR and PAR production), but errors bars in Fig. 5 are very small relative to those in Fig. 4 (they should be larger). I recommend using propagation of errors to

calculate the variance of % Inhibition (see supplemented pdf).

Reference:

Obernosterer, I., Herndl, G. J. (1995) Phytoplankton extracellular release and bacterial growth: dependence on the inorganic N: P ratio MEPS 116:247-257

Please also note the supplement to this comment: http://www.biogeosciences-discuss.net/9/C3659/2012/bgd-9-C3659-2012supplement.pdf

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