Reviewer #2

Wu et al. present a study of the photophysiological responses of two diatoms as affected by the temperature during exposure. The responses are observed during short-term exposures to high light (with and without UV) and subsequent recovery periods in low light. By tracking the kinetics of PSII quantum yield during the treatment, inferences can be made about the relative contribution of damage and repair processes to the variations in response between temperature. Additional information can be obtained by exposing the diatoms in the presence of the repair inhibitor lincomycin. This type of approach has been in previous studies of how variation in environmental factors influence inhibition and recovery kinetics, however most studies have focused on a single time scale of treatment, usually on the order of hours to a few days. This study is distinctive in comparing the response to a short-term increase in temperature to responses for cultures acclimated over some growth period to the same temperature. One detail that should be added, however, is how long the acclimated cultures were maintained at their growth temperature before the experiment.

Response: We appreciated the comments very much. The culture was maintained at 3 temperature levels for 5 days before the experiment. For the acclimation time, we have added information at lines 125-127.

In general, the authors do a good job of presenting the experimental approach and results. I list below some specific comments that should be addressed. I think the discussion could do a better job of putting the results on damage and repair rates in the context of other studies. How do these diatoms compare with other taxa that have been studied and what does that say about their (relative) resistance to PAR and UV inhibition? One study that is not referenced is that of Sobrino et al. (2007) which examined the responses of the centric diatom, Thalassiosira pseudonana following a similar approach as used in the present study, i.e. comparing the effects of both short-term and long-term shifts in temperature. Sobrino et al. found that moderate short-term increases in temperature increased damage and repair rates but both rates decreased with long-term acclimation to the same temperature. It would be interesting

for the authors to compare their results with this previous study. One conceptual difference with the present study is that Sobrino et al., on the basis of exposure-response curves, base their kinetic determinations on an equation that assumes that repair operates at a fixed rate due to an apparent saturation of repair rate at high rates of damage. This equation is:

$$P = \left(\frac{r}{k} + \frac{r-k}{r}\right) * e^{-kt}$$

Here "P" represents relative rate as a function of time (cf. Pt/P0). This differs from the Kok equation (the author's equation Line 168) which assumes that the contribution of repair to the active pool is proportional to damage. Which equation is used does have implications for the inferred repair rate which will have different implied units depending on which equation is used, the rate is specific to the pool size of damaged "sites" for the Kok equation but is an absolute rate, fraction of pool repaired with time, for the Sobrino et al. equation. So the rates can't be directly compared, but the patterns of variation with temperature can.

Response: We appreciated the comment, and have read the paper by Sobrino et al., (J. Phycol.2007). One of the main findings in that paper, i.e. "temperature and UVR interact mainly over short (hours) rather than long (days) timescales" offers strong support for present study, since we mimicked the short term increase of temperature likely to be experienced on an intertidal flat, and our data indicated that temperature was a very important factor in influencing microphytobenthos. In addition, the findings of Sobrino et al. on the relationship between BWF and dynamics of repair versus damage was interesting. We have made substantial changes in the discussion. We have not, though, run our data through the Sobrino et al. equation, sticking to the Kok equation for our analysis; as the reviewer states this will not allow absolute rates to be compared but the patterns of variation with temperature will be comparable.

If further studies are performed on these species, it would be informative to examine different exposures and see if the exposure-response curve is better fit using the model with repair increasing over the full range of exposure (Kok model), or whether repair "saturates" to a fixed rate as for *T. pseudonana*. The latter situation has been

generalized into the *E*max model (Neale et al. 2014), which seems to be broadly applicable to marine phytoplankton.

Response: We agree with the reviewer that a comparison with different models is required for future studies. We have additional data on several *Thalassiosira* species that encompass a wide range of size, we hope that we can do a comparison with previous work in our next step.

Specific Comments:

Culture: As mentioned, specify how long cultures were maintained at each temperature before the experiment.

Response: Added as suggested.

Semi-continuous growth – how often were cultures diluted? Growth rates-Methods to determine growth rate (tracking of F0-fluorescence, lines 115-118) more appropriately included with culture conditions section. Specify what was the time interval between T1 and T2. Were multiple determinations made of growth rate for each replicate culture?

Response: The culture was diluted every day with fresh medium. We have added this information at lines 107-108. We have moved the growth rate section into the culture conditions section at lines 111-113, the time interval between T1 and T2 was one day.

Spectra: Line 114-115 discussion of filter transmission is out of place, add to Experimental set up where the cut-off filters are described.

Response: We have moved this sentence to lines 136-137.

Experimental set up: No information was available on the internet for the radiometer used, please a specific source or details filter type, bandwidth, calibration, etc. Note that a 280 nm cutoff in conjunction with a Xenon lamp means that the samples are being exposed to some irradiance at wavelengths < 290 nm which do not occur under natural solar exposures.

Response: The radiometer was produced by a domestic company (http://www.tinel.cn/). The bandwidth of the filters for UVA and UVB were 315-400 nm and 280-315 nm, the radiometer was certified by National Institute of Metrology, China. The sensitivity of this radiometer for UVA and UVB was 0.1 and 0.01 W m^{-2} respectively, and is

somewhat lower than the radiometer that we have used before (ELDONET), but was sensitive enough for the present work. We have added specific source information about this radiometer at line 127.

We agree that the intensity of wavelengths <290nm is negligible at the surface of the Earth. However, because we also want, in future, to run experiments to evaluate the spectral sensitivity of diatoms (and construct biological weighting functions), we used a 280 nm filter here to have a better comparison with future work.

Temperature change: A 10 deg shift could occur in the intertidal benthic environment, but this is not a change that *Skeletonema* is likely to encounter

Response: We agree with the reviewer that a 10 ^{o}C rise is unlikely for planktonic species, however, the purpose of our study was to compare species from different niches, so *Skeletonema* here is more likely a reference species. In addition, we have 3 growth temperatures with a 5 ^{o}C increase, which could be applicable to coastal phytoplankton.

Chlorophyll fluorescence: It is stated that yield measurements were made on subsamples withdrawn from the treatment tubes. What was the light condition during measurement – I'm guessing it was low or dark. Also, was there a dark adaption period before measurement? If the measurement is not on the sample in treatment irradiance, what is measured is not an effective yield under actinic light, different from what is stated on lines 154-156. Instead the steady-state fluorescence is (or is close to) F0', minimal fluorescence in the presence of nonphotochemical quenching (NPQ) which persists after highlight exposure (depending on the extent of dark adaptation), and the yield is the maximal (or intrinsic) yield. Maximal yield (not dark adapted) will reflect the induction and dissipation of NPQ as well as changes in functional PSII.

Response: The reviewer is correct that the sub-sample experienced a very short-term dark period (<20 seconds) before measurement of chl fluorescence. Strictly speaking, our measurement was not effective yield, nor the dark-adapted value (which requires at least 15 min darkness). However, based on our experience with diatoms that are

exposed to high light/UV, the yield of PSII recovered much slower under darkness than under low light conditions (Wu et al., 2014, J. Photochem. Photobiol. B). So although the value measured in the present work was not perfect, it should be a reasonable operational proxy. To avoid misleading readers, we have reworded the statement at line 159-165.

Data Analysis: How was "k" estimated from lincomycin treated results – fit to an exponential curve? For both the "k" and "r" fits, statistics should be reported on the standard error of the parameter estimates (available from most non-linear regression routines) and R2 of the fit. In some of the cases of UV exposure, it does not appear as though the Kok equation would give a very good fit as the yield never stabilizes to a steady-state (e.g. results from 15 deg exposures). In these cases, the uncertainty in parameter estimates will far outweigh the variability associated with replication.

Response: For the k estimation from the lincomycin treatment, we fitted the lincomycin data into the Kok model with r fixed as zero (when the equation will be $Pt/P0=e^{-kt}$), so it is an exponential curve. We agree with the reviewer that the data fit for some treatments was not good, and resulted in higher standard deviations for some data points. For the quality of the fitting, we summarized r square values in a table as supplementary information (also see below), and hope this could be of help for the reader. We also added related information in the results section.

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Table A3 R square values for curve fitting with the Kok model for independent replicates of the two species under different temperature and radiation treatments

Species	Radiation treatment	replicate No.	Temperature treatment (°C)					
			15	15-25	20	20-30	25	25-35
Skeletonema sp.	P	1	0.98	0.85	0.74	0.72	0.93	0.96
	P	2	0.96	0.97	0.73	0.82	0.96	0.96
	P	3	0.97	0.89	0.80	0.75	0.98	0.97
	PAB	1	0.91	0.94	0.92	0.97	0.97	0.99
	PAB	2	0.94	0.95	0.87	0.94	0.96	0.97
	PAB	3	0.95	0.85	0.91	0.98	0.92	0.99
Nitzschi	P	1	0.77	0.84	0.78	0.96	0.87	0.98
	P	2	0.74	0.89	0.75	0.93	0.82	0.96

P	3	0.74	0.84	0.73	0.86	0.88	0.90
PAB	1	0.99	0.97	0.98	0.97	0.87	0.86
PAB	2	0.98	0.93	0.95	0.95	0.89	0.86
PAB	3	0.97	0.96	0.96	0.97	0.93	0.88

Line 186: While … Not a sentence, no verb

Response: As suggested by reviewer 1, we have deleted this paragraph.

Lines 222-225 Not clear what is meant by a "similar pattern". The decrease in yield in the presence of lincomycin is obviously much greater due to the presence of the inhibitor

Response: Thanks for the comment, we have reworded this sentence.

Line 229-230 – In the range.. Not a complete sentence

Response: Reworded

References:

Sobrino, C., and P. J. Neale. 2007. Short-term and long-term effects of temperature on phytoplankton photosynthesis under UVR exposures. J. Phycol. **43:** 426-436.

Neale, P. J., A. L. Pritchard, and R. Ihnacik. 2014. UV effects on the primary productivity of picophytoplankton: biological weighting functions and exposure response curves of *Synechococcus*. Biogeosciences **11:** 2883-2895.

Response: Thanks for the references, we have cited them in the appropriate places (e.g. line 295-296, 333-334).