Reviewer #1

This paper presents results from laboratory experiments manipulating the UVR (two levels) and temperature to assess the sensitivity of two diatom species to both factors. The experiment was performed during 120 minutes a single time. The study deals with an interesting topic to phytoplankton ecologists, and tries to clarify a relevant question on the differential photosynthetic responses of the benthic and planktonic species in coastal areas against a scenario of global warming.

Response: We appreciate the comments very much; we would like to make a clarification here that the primary purpose of this study was to test the hypothesis that benthic diatoms have a stronger ability to cope with stressful solar UV radiation under the high temperature regimes that are frequently experienced by benthic species on intertidal flats.

However I find several problems in the manuscript:

The first impression after reading this manuscript is that it is rather long for the type of study done. The topic is interesting, but this is really a snapshot experiment on two hours on two diatoms species. The most suitable presentation of these results would be/could be as a Note and not as a full length paper. On the other hand, this very short-term experiment, with increments of 10 C in temperature, is very unrealistic. Furthermore, a conclusion like this; "the temperature-mediated UV sensitivities might also have implications for phytoplankton in the future warming oceans" seems to me too much speculative.

Response: We agree with the reviewer that the experiment involves short-term light exposure, however, we would argue that in some situations this actually reflects the scenario in the natural environment: the microphytobenthos are often exposed to the coupled stresses of high light and high temperature over a short-term time scale (e.g. during low tide emersion). In addition, we also acclimated both species under different temperatures for at least 5 days before the UV treatment, so that we have data on both short-term and long-term increases of temperature. We believe that this manuscript raises interesting questions that need to be tested more rigorously on a longer time scale under UV radiation, as well as with a broader range of benthic and planktonic species.

For the temperature manipulation, the present manuscript focused on the likely temperature increase on the intertidal flat during low tide periods, rather than mimicking a future scenario of global warming. As measured by Laviale et al., (2015, Environmental Microbiology), the in situ temperature change on the intertidal flat can be greater than 10 ºC. Therefore, the simulation of temperature increase in this work is close to what happens in the natural environment. We realized that the last sentence in the abstract might confuse the reviewer that we are dealing with a global warming issue, and this has been deleted in the revision.

My main concern is related to the statistical analysis performed in this study which is not suitable to the experimental design performed and to test the working hypothesis. The authors manipulated two independent factors, so they should do a two-way ANOVA. Also, when authors analysed the effect on variation in the time of the photosynthetic response to light and dim, they should use a RM-ANOVA. Only when they evaluated the temperature effect on the relative UVR inhibition (%), one-way ANOVA is the correct statistical procedure. Moreover, to test their hypothesis, the authors should evaluate the interactive effect UVR and temperature on the two species as well as to quantify the magnitude of these interactive effects. To my impression a wrong test was used. This fatal error determines that the results and discussion must be re-written.

Response: As suggested by the reviewer, we have done this statistical work and found that UV affected both species significantly under all temperature levels except for *Skeletonema sp.* under 35 ºC. While the interactive effects of temperature increase and UV were significant for *Skeletonema sp.* over the full range of temperature, and interactive effects were found for *Nitzschia sp.* when temperature increased by 10 ºC from 15 or 20 ºC; however, no interactive effect was found for the highest temperature (25-35 ºC), which we take as strong evidence that *Nitzschia sp.* was relatively resistant to the coupled stresses of high temperature and UV radiation. We have incorporated these results into the revision.

The estimation of the growth rates is confusing. From the description done, it is not easy to understand how was calculated. If I have understood, it was calculated on

fluorescence variation in a 1-hour interval of time, so unit cannot be day; Moreover, I think that the fluorescence is not a good proxy of biomass or abundance, therefore these values did not represent an accurate measurement of growth rates; caution should be taken to discuss this result with those from literature generally obtained from changes of biomass or abundance.

Response: We are sorry that the description about growth rates was not clear. In fact, we measured the fluorescence change over 1 day intervals. As a proxy of biomass or abundance, the most direct estimation is cell counts, POC or *in vivo* chl *a*. Kruskopf and Flynn (New Phytologist, 2005) argued that chlorophyll fluorescence is questionable for biomass estimation of phytoplankton, especially for cultures under nutrient depletion. However, their results actually showed a good correlation between in vivo chl *a* and fluorescence for cultures with relatively lower biomass, <0.25mg chl *a* L^{-1}, as the was the case for the cultures in the present study (<0.02mg L^{-1}). Consequently, we believe chl *a* is a robust proxy for growth under our experimental set up.

In the results section, there is a lack of precision in the description of the results, making them difficult to understand. The authors should consider remove some of the figures (e.g. Fig. 1 and Fig 2). I think that the figures should be regrouped in two panels, one per each specie, it could benefit the understanding of the Ms. You should present the results in a more synthetic way.

Response: Thanks for the comments, we have moved Fig 1 and Fig 2 into supplementary information. For the arrangement of figures, the primary purpose of our study was to compare species from different niche environments, so we would like to keep the present arrangement with 2 species in one figure, for a better comparison between the two species. We have however made substantial changes to the results section in order describe the data more precisely.

I would like to see the results of the statistical analysis in tables, with the df, F and p values. Likewise, the post hoc results should be presented as part of the figures (lowercase letters).

Response: We have summarized the statistical results as Table A1 and Table A2 in the

supplementary information (also see below), and have also indicated the significance in Fig 4-7 with lowercase letters.

Table A1 The statistical results of RM-ANOVA for the comparison of effective quantum yields under P and PAB at a single temperature level

species	Temperature type	Temperature level (°C)	df	F	p
Skeletonema sp	Acclimated	15	5	30.12	0.000
		20	5	8.89	0.000
		25	5	11.38	0.000
	Short term	25	5	9.78	0.000
		30	5	3.05	0.033
		35	5	0.74	0.604
Nitzschia sp	Acclimated	15	5	38.76	0.000
		20	5	10.09	0.000
		25	5	13.28	0.000
	Short term	25	5	11.85	0.000
		30	5	9.96	0.000
		35	5	5.42	0.003

Table A2 The statistical results of RM-ANOVA for effective quantum yields during light exposure under different temperature and radiation treatments.

Species	temperature increase	Factors	df	F	p
Skeletonema sp	15-25	time	5	431.0	0.000
		time*temperature	5	39.43	0.000
		time*light	5	36.17	0.000
		time*temperature*light	5	2.98	0.022
	20-30	time	5	532.46	0.000
		time*temperature	5	7.85	0.000
		time*light	5	6.39	0.000
		time*temperature*light	5	4.35	0.003
	25-35	time	5	1127.84	0.000
		time*temperature	5	135.11	0.000
		time*light	5	6.76	0.000
		time*temperature*light	5	2.46	0.049
Nitzschia sp	15-25	time	5	742.92	0.000
		time*temperature	5	19.46	0.000
		time*light	5	40.5	0.000
		time*temperature*light	5	2.5	0.046
	20-30	time	5	816.48	0.000
		time*temperature	5	11.12	0.000

	time*light	5	16.77	0.000
	time*temperature*light	5	3.26	0.015
25-35	time	5	299.57	0.000
	time*temperature	5	4.16	0.004
	time*light	5	17.15	0.000
	time*temperature*light	5	1.61	0.178

The authors should pay attention to repetition through the text of terms which was defined in M&M (for instance, photosystem II (PSII), damage rate (k) repair rate (r), Effective quantum yield (y) etc… Likewise, the authors should be consistent with the name of treatments (P exposed not PAR-exposed; UVR vs PAB) through the text; and in figure legends the radiation treatments are written as P or P+UVR whereas in graphs are shown as P and PAB. Finally, the variables should be clearly defined, (e.g. Relative UV inhibition (%) in figures but in line 159 Relative inhibition (%) etc…).

Response: Thanks for the comments, we have revised the text accordingly throughout the manuscript.

Specific comments

Abstract

It is Ok

Response: No response needed here.

Introduction

Line 85-90. This paragraph might seems repetitive.

Response: We have reworded this paragraph.

Method:

Using the Aquapen fluorometer the authors had to remove 4 ml for each measurement (I'm assume that the cuvette is 1 cm), there are 5 measurements in light, 5 in dim plus an initial sample, so in sum about 45ml are needed. How this work if the sample volume had only 35ml?. This needs to be clarified.

Response: The reviewer is correct that the full volume of cuvette is around 4 ml, while during the experiment, we withdrew 2 ml for measurement (which was shown to be adequate by preliminary tests). So a 35 ml sample is enough for the whole experiment.

We have added information to this effect at line 144.

Line 104. both species were inoculated into enriched seawater… It would be necessary to give more details about the culture medium, please.

Response: The medium recipe was Aquil; we have added this information at line 104.

Line 110. Determination of spectra, What do you mean?

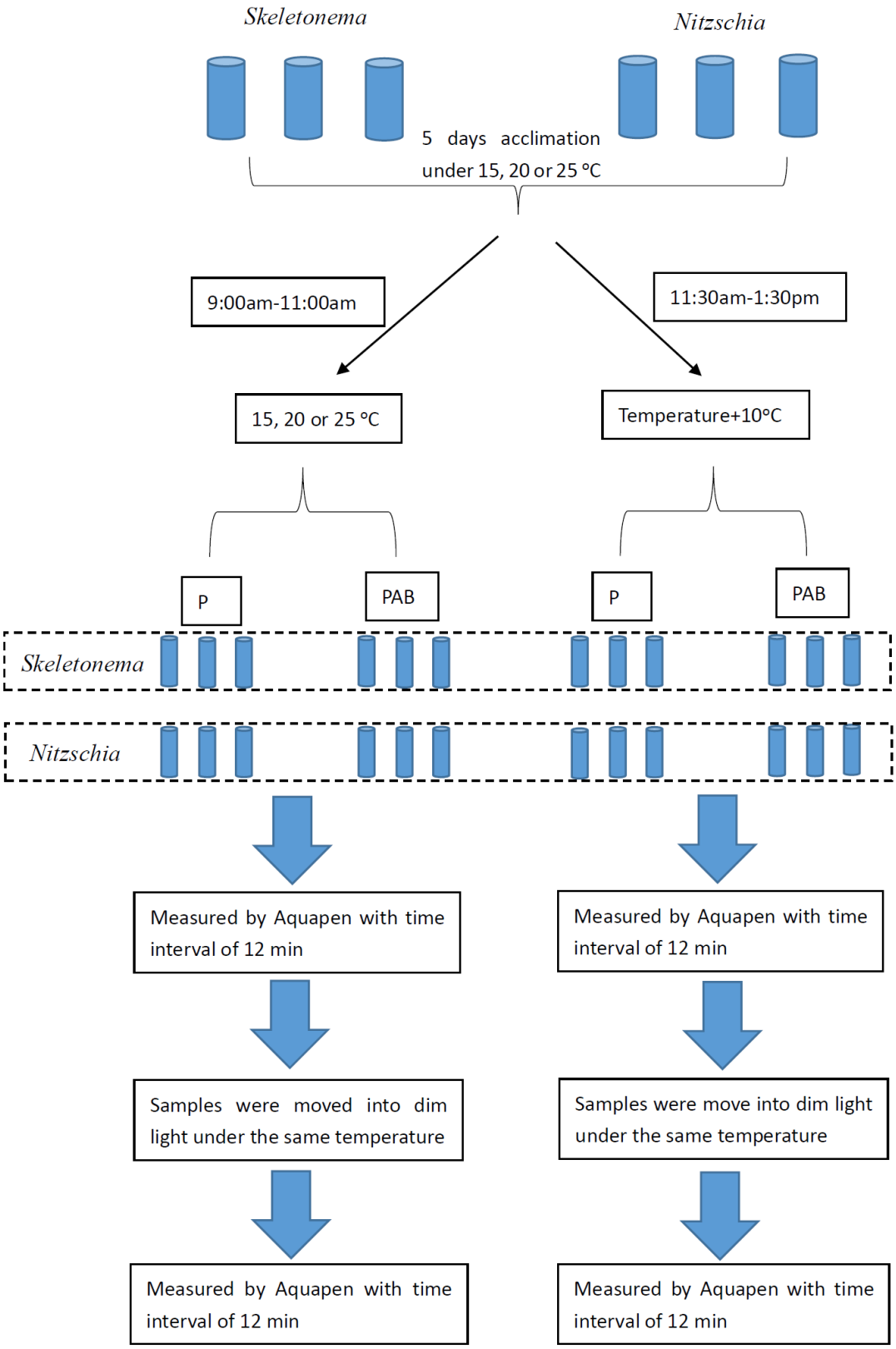
Response: Sorry for the confusion, we determined the absorbance spectra of extracted pigments as well as the transmission spectra of cut-off filters. We have reworded this paragraph.

Line 114 . This sentence The cut- off filters were scanned in the same wavelength range against air as a blank. I think it is not the suitable place, because it makes the text confusing.

Response: As suggested by both reviewers, we have reworded this sentence and moved it to line 136-137.

Line 141. A total of 12 tubes (2 species and 2 radiation treatments)…..? The temperature treatments were not made simultaneously? Moreover, how were done the measured of acclimated vs. short-term samples? I can´t understand how the experiment was performed. I hope to be wrong, but seems that the experiment was not a full factorial. In my opinion, the paper would benefit if an illustration of the experimental design would be included.

Response: We have two species under two light treatments (P, PAB) and six temperature treatments, and triplicates for each species so in total we had 2*2*6*3=72 tubes. It is impossible to run all treatments simultaneously, especially for present study to track the kinetics of PSII activity. We then maintained the culture at exponential phase by dilution with fresh medium every day, to keep a stable physiological status, and took samples in the middle of the light period for temperature (2 levels, acclimated, or acclimated+10 ^{o}C) and light treatments (P, PAB). We have reworded the appropriate sentences in the M&M and added an illustration (Fig A2) in the supplementary information (shown below).



Line 169. This sentence “where P0 and Pt represent the initial effective quantum yield

and yield at time zero and t (minutes), respectively" is confusing, perhaps is better …… where P0 and Pt represent the effective quantum yield at time zero and t (minutes), respectively.

Response: we have reworded this sentence at suggested.

The propagation errors should be applied to calculate the variance of the relative inhibition UVR (as percentage) as well as the variance in the quotient r:k

Response: We thank the reviewer for this reminder to take into account error propagation; we have now calculated the variance for relative UV inhibition and the quotient r:k, and combined these values as error bars in Figure 4, 5 and 6.

Results

Lines 181-186. This paragraph should be removed because the data are not very informative.

Response: As suggested, we have removed this paragraph and also the related figures.

Line 222-225. I'm sorry, but I don't reach to see what brings to this study the treatments with antibiotic.

Response: For the study of repair/damage of PSII, lincomycin is often used to block the repair process, to get a better estimation of rate constant for damage. We have reworded the sentence at line 127-129, to present the purpose of using the antibiotic more clearly.

This section presents comparisons among different temperatures and radiation treatments which could not be evaluated by one-way ANOVA, and post hoc analysis, except to the relative inhibition UVR variable. See above

Response: We have reanalyzed these data by RM-ANOVA, and added p values and F values in this section, and reworded the sentences as necessary.

Discussion

Line 260-264. This paragraph is very general; I would like to read something about what is the main contribution of this study.

Response: We have reworded this paragraph as suggested.

The discussion, probably will be modified after addressing the points and questions related with experimental set-up and statistical analysis.

Response: We have made substantial changes according to the new statistical results.