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 added these values in the results section as necessary, we 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 160.

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 112.

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

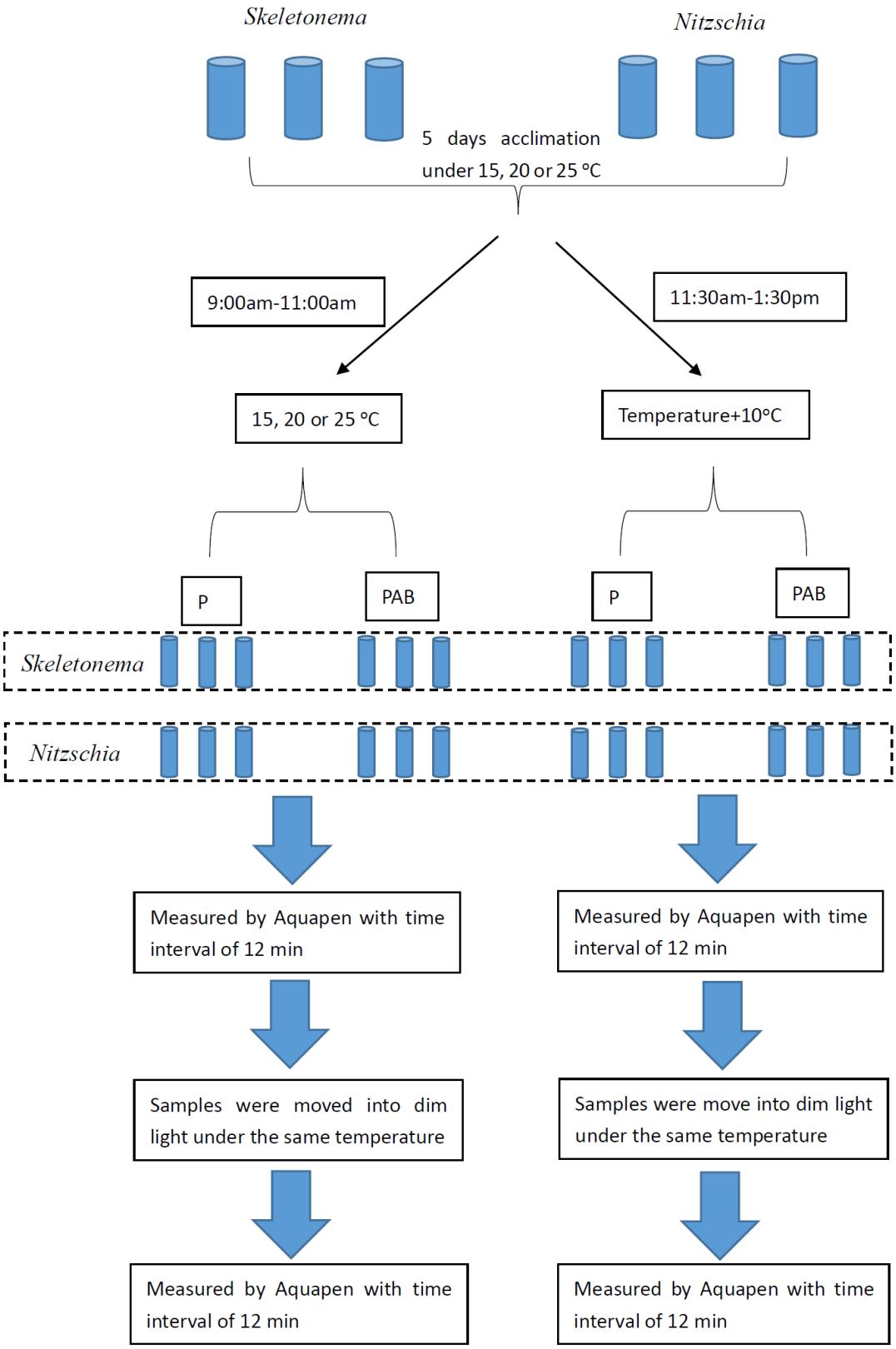
Response: Sorry for the confusion, we determined the absorbance spectra of extracted pigments as well as the transmission spectra of cut-off filters. We have reworded this paragraph at line 123-131.

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 145-147.

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 line 190-191.

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 141-143, 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 at line 303-314 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.

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 137-139.

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 (e.g. at line 328-331, 349-350, 370-374 etc.). 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 119-121, 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 145-147.

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 136.

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 ºC 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 ºC 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 169-175.

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.

.

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

Nitzschia sp	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 at line 262-265.

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. at line 331, 350, 373 etc.)

Differential photosynthetic responses of marine planktonic and benthic diatoms to ultraviolet radiation under various temperature regimes

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Abstract:

We studied the photophysiological responses to ultraviolet radiation (UVR) of two diatoms, isolated from different environmental niches. Both species showed the highest sensitivity to UV radiation under relatively low temperature, while they were less inhibited under moderately increased temperature. Under the highest temperature applied in this study, the benthic diatom *Nitzschia sp.* showed minimal sensitivity to UV radiation, while inhibition of the planktonic species, *Skeletonema sp.*, increased further compared with that at the growth temperature. These photochemical responses were linked to values for the repair and damage processes within the cell; higher damage rates and lower repair rates were observed for *Skeletonema sp.* under suboptimal temperature, while for *Nitzschia sp.*, repair rates increased and damage rates were stable within the applied temperature range. Our results suggested that the response of the microalgae ~~phytoplankton~~ to UV radiation correlated with their niche environments, the periodic exposure to extreme temperatures ~~promote~~ promoting the resistance of the benthic species to the combination of high temperature and UV radiation. ~~Furthermore, the temperature mediated UV sensitivities might also have implications for phytoplankton in the future warming oceans.~~

Keywords: Diatom, Photosynthetic performance, Temperature, UV radiation

Introduction

As the most abundant group of ~~phytoplankton~~microalgae, and one that plays an important role in marine ecosystem function and biogeochemical cycles, diatoms are traditionally divided into centric and pennate species on the basis of their valve symmetry (Round et al., 1990). Centric diatoms are usually, though not invariably, planktonic and pennate species are benthic, and are often found living in different niches (Irwin et al., 2012; Keithan et al., 1988). The distribution of centric diatoms is more widespread, with records for the open ocean as well as coastal water, and they maintain their position in the upper mixing layer by maintaining buoyancy with elaborated spines or excretion of heavy ions (Lavoie et al., 2016;Villareal, 1988). In contrast, pennate diatoms are often found in the intertidal zone (Stevenson, 1983). Therefore, the 2 groups of diatom are likely to have evolved different strategies to cope with their niche environments (Barnett et al., 2015;Lavaud et al., 2016;Lavaud et al., 2007).

Temperature affects almost all biochemical reactions in living cells, and is one of the most important factors that determines the biogeography, as well as the temporal variation of phytoplankton (Levasseur et al., 1984). Under global change scenarios, increases in sea surface temperature would re-structure the phytoplankton assemblages in the future ocean (Thomas et al., 2012). At small spatial scales, e.g. the coastal zone, diurnal cycle of tides or meteorological events could expose benthic diatoms to extreme environments, including high photosynthetically active radiation (PAR) and ultraviolet (UV) radiation ~~(UV)~~ exposure as well as larger variations in temperature than found for planktonic species. Hence organisms in such exposed areas should potentially possess highly efficient mechanisms to adapt such environment (Souffreau et al., 2010;Weisse et al., 2016).

In the intertidal zone, UV radiation (UVR) is another driving force. UVR is a component of the solar spectrum, along with photosynthetically active radiation (PAR), and has wide reaching effects on organisms, especially photoautotrophs due to their demands for light energy (Williamson et al., 2014). The penetration of effective UVR

in coastal waters is mainly dependent on the properties of the seawater (Tedetti and Sempere, 2006). Previous studies have found that UVR significantly inhibited carbon fixation by phytoplankton in the surface layer, with less inhibition or even stimulation in deep water due to low UVR and limiting levels of PAR (Gao et al., 2007). Detrimental effects, however, varied seasonally, with less inhibition observed for planktonic assemblages during summer, though UV radiation was the highest. This may be attributable to the higher water temperature which facilitated enzyme-catalyzed repair processes within the cell (Wu et al., 2010). There are few documented studies on benthic species, which actually are potentially more resistant to UVR as they are periodically exposed to high solar radiation during low tide (Barnett et al., 2015).

Photosystem II (PSII) initiates the first step of photosynthesis, converting photons to electrons efficiently, but this complex is very sensitive to light (Campbell and Tyystjarvi, 2012). The subunits of PSII are broken down under UVR or high PAR while repaired by insertion of de-novo synthesized protein (Aro et al., 1993); the repair process eventually reaches a dynamic balance with damage (Heraud and Beardall, 2000). However, these two processes are independent from each other. The photochemical damage is mainly determined by the intensity and spectrum of light (Heraud and Beardall, 2000) and is temperature insensitive, while the repair process is driven by a series of enzyme-catalyzed reactions, and is thus potentially sensitive to temperature changes (Melis, 1999). Previous studies revealed that high temperature alleviated UV inhibition of ~~photosystem~~ PSII in green algae (Wong et al., 2015), while it interactively decreased photosynthetic activity in microphytobenthos under excessive PAR conditions (Laviale et al., 2015).

~~Coastal water is a highly productive zone, with most of primary productivity attributed to diatoms~~ Considering the importance of diatoms to coastal primary productivity (Carstensen et al., 2015)~~.~~, their responses to ~~Hence, how diatoms respond to~~ environmental factors~~, e.g. UV radiation, nutrient pulses or temperature, has been extensively studied~~ ~~aroused broad~~are of considerable interest~~s~~ (Häder et al., 2011). ~~These responses were often shown to be species-specific, and could correlate with cell~~

~~size, geometry or distinct mechanisms operated by different species (Halac et al., 2014;Wu et al., 2015). Considering~~ However, the niches in which planktonic and benthic diatom species ~~are living~~exist~~,~~ ~~e.g. physical and chemical factors, are~~have quite different physical and chemical ~~quite different between planktonic and benthic species~~characteristics (Souffreau et al., 2010). In this study, we ~~will use~~used two freshly isolated ~~isolated~~ species to test the hypothesis that benthic diatoms have a stronger ability to adapt to potentially stressful solar UV radiation under high temperature regimes.

Materials and methods

1. Species and culture conditions

We collected samples from offshore water and intertidal sediments in the coastal area of the Yellow Sea. These were re-suspended in seawater, and enriched with Aquil medium and incubated in a growth chamber for 3 days (Morel et al., 1979). Then a sub-sample was examined under a microscope, and single cells were picked up with a micro pipette. *Skeletonema sp.* and *Nitzschia sp.* were chosen for the present study, and were maintained in Aquil medium in a growth chamber at 15 °C. Prior to the experiment, both species were inoculated into enriched seawater (Aquil medium) and cultured semi-continuously in 500 mL polycarbonate bottles, illuminated with cool fluorescent tubes at a photon flux density of ~200 μmol m^{-2} s^{-1}, with a 12:12 light/dark cycle. ~~While~~ Tt~~t~~emperature was set at 15, 20 or 25 °C~~-~~, with variation less than 0.5 °C, and cultures were diluted every day with fresh medium~~, b~~. Bottles (triplicates for each temperature) were manually shaken 2–3 times during the light period and randomly distributed in the growth chamber.

Specific growth rate was estimated from the changes of dark adapted chlorophyll fluorescence (see below), and calculated as: $\mu = (\ln F_2 - \ln F_1) / (D_2 - D_1)$, where F_1 and F_2 represent the steady-state fluorescence intensity at day 1 or day 2, respectively.

2. Determination of the absorption ~~the~~ spectra of pigments~~ectra and growth rate~~

50 mL of culture was filtered onto a GF/F filter, and extracted in 5 mL absolute methanol for 2 h at room temperature in a 10 mL centrifuging tube, then centrifuged at 4000 rpm for 15 min (TDZ4-WS, Luxiang Inc.). The supernatant was scanned with a spectrophotometer (Lambda 35, PerkinElmer) in the range of 280nm-750 nm. ~~The cut-off filters were scanned in the same wavelength range against air as a blank. Specific growth rate was estimated from the changes of dark-adapted chlorophyll fluorescence, and calculated as: $\mu = (\ln F_2 - \ln F_1) / (T_2 - T_1)$, where F_1 and F_2 represent the steady-state fluorescence intensity at T_1 or T_2, respectively.~~

3. Experimental set up

The experiments were performed under a customized solar simulator with a 1,000 W xenon arc lamp as the light source. The incident irradiances of UV-B light (280–315 nm), UV-A (315–400 nm), and PAR (400–700 nm) were measured using a broadband radiometer (SOLAR-2UV, TINEL Inc.~~,~~ *http://www.tinel.cn*~~/~~).

After 5 days acclimation under the target temperature, ~~In the middle of the light period,~~ samples of both species in the exponential phase were harvested during the middle of the light period, and directly transferred to quartz tubes (35 mL) at a density of less than 20 μg chl *a* L^{-1}, dark-adapted for 15 min, and ~~added~~treated by addition of ~~with~~ milli-Q water (as a control) or lincomycin (final concentration, 0.5 mg mL^{-1}~~,~~); the latter inhibits protein synthesis and was used ~~for the~~to get a better determination of damage rate in the absence of repair~~) were added~~. The tubes were then placed into a water bath one after another at 1 minute intervals while covered with cut-off filters (ZJB280, ZJB400) that block radiation below 280 or 400 nm, respectively (the filters ~~was~~properties were checked by scann~~ed~~ing in the wavelength range of 280-750 nm against air as a blank~~, 50% transmission at 280 nm or 400 nm~~, see Figure A1), to create PAR + UV-A + UV-B (PAB) and PAR (P) treatments respectively. The light levels applied were PAR =440 μmol photons m^{-2} s^{-1} and UVR = 41.6 W m^{-2}, while temperature was controlled with a cooling system (CTP3000, Eyela) and was set as the incubation level (termed "acclimated") or the incubation temperature +10 °C (termed "short term"), the latter mimicking a moderate increase in temperature in the intertidal zone during a

low tide period. After the light exposure, samples were moved into a water bath at the same temperature as light exposure, but under dim light (~30 μmol photons m^{-2} s^{-1}), for recovery, effective quantum yields were then measured at 12 min intervals. ~~D~~The detailed experimental design can be found in Fig A2~~1~~ in the supplementary information.

4. Chlorophyll fluorescence measurements

A total of 12 tubes (2 species and 2 radiation treatments for ~~one~~each temperature level) were dark-adapted for 15 min, then each tube was moved into a water bath one by one ~~with~~ at 1 minute intervals for light exposure, and 2 mL sub-samples were taken to measure the initial chlorophyll fluorescence with an Aquapen fluorometer (AP-C 100, PSI). During the subsequent light exposure, sub-samples were withdrawn every 12 minutes from the quartz tubes for fluorescence measurement~~,~~; this procedure ensured that every sample was exposed to radiation ~~with~~ for exactly the same time ~~duration~~. After five rounds of measurements (60 min), samples that were without lincomycin were transferred into the low light condition under the same temperature for recovery, and chlorophyll fluorescence was measured as above for 60 min.

5. Data analysis

Effective quantum yields were measured after 20 s of dark period~~ness~~ (operational time between sampling and measuring) with the AquaPen and calculated according to the following equations:

Effective quantum yield = $(F_m' - F_{o,t}') / F_m'$

where F_m' is the effective maximal fluorescence, and F_o'~~F_t~~ is the minimal fluorescence in the presence of nonphotochemical quenching which persists after highlight exposure~~steady-state fluorescence under actinic light~~.

The relative UV inhibition of effective quantum yield ~~by UV~~ was estimated according to the following equation:

Relative UV inhibition (%) = $(P_P - P_{PAB}) / P_P \times 100$,

where P_P and P_{PAB} represent the effective quantum yield under P and PAB treatments, respectively. Relative UV inhibition was calculated when P_P and P_{PAB} were significantly different. ~~, propagation errors were applied to calculate the variance of inhibition (in~~

~~percentage).~~

The rates of UVR-induced damage to ~~photosystem~~ PSII ~~(PSII)~~ (k, min^{-1}) were calculated from lincomycin treated samples assuming repair (~~r~~) under these conditions was zero. Repair rates (r, min^{-1}) were calculated using non-lincomycin-treated samples with the fixed k values obtained from the parallel experiments with lincomycin. Both calculations were made according to the Kok equation (Heraud and Beardall, 2000):

$$\frac{P_t}{P_0} = \frac{r}{k+r} + \frac{k}{k+r} e^{-(k+r)t},$$

where P_0 and P_t represent the effective quantum yield at time zero and t (minutes), respectively. ~~For the ratio of r to k, propagation errors were applied to calculate the variance. where P_0 and P_t represent the initial effective quantum yield and yield at time zero and t (minutes), respectively.~~

The recovery rates under dim light were calculated with a simple exponential rise equation (Heraud and Beardall, 2000)~~.~~:

$$y = y_0 + c\,(1 - e^{-\alpha t})$$

where y represents the effective quantum yield at time t (minutes) during the dim light incubation, α was the recovery rate, while y_0 and c are constants.

Statistical differences for the kinetics of changes in effective quantum yield~~s~~ among treatments were analyzed with ~~a one-way~~repeated measures analysis of variance (RM-ANOVA). ~~and While t~~The differences of relative UV inhibition and rate constants among treatments were analyzed by one-way ANOVA~~,~~; ~~Tukey HSD was conducted for *post hoc* investigation. A~~a confidence interval of 95% was set for all tests. For the calculation of the ratio of r : ~~,~~k and the relative UV inhibition –(%), propagation errors were taken into account to estimate variance.

Results

~~*Skeletonema sp.* had a lower growth rate under 15 and 20 °C (p<0.05), while~~

~~growth increased significantly and was 23% higher than that of *Nitzschia sp.* under 25 °C (Fig 1) (p<0.01). The spectra of methanol extracts of both species had a similar pattern, *Nitzschia sp.* showed relatively higher absorption in the range of 410-480 nm under 15 or 20 °C (Fig 2 A, B), and this further increased significantly under 25 °C (Fig 2C). While no obvious peak in the UV range for both species.~~

The initial photochemical quantum yield of ~~15 °C grown~~ *Skeletonema sp.* grown at 15 °C was around 0.50 during light exposure (incubated under 15 °C), but decreased gradually toward the end of the radiation treatments, with lower values under PAB than under the P condition (p<0.001, F=30.1) (Fig 1~~3~~A, Table A1). During the dim light exposure period, the quantum yield recovered to its initial value within 24 min under P treatment, while PAB treated cells only recovered partially to ~70% by the end of the dim light incubation (Fig 1~~3~~A). For 15 °C grown cells that were incubated under 25 °C, the general patterns were similar ~~as~~ to those incubated under 15 °C~~,~~; ~~though with smaller~~ the differences between the P and PAB treatments was smaller but still significant (p<0.001, F=9.8) (Fig 1~~3~~B, Table A1). Under dim light, the quantum yield of cells under both radiation treatments recovered to near initial values (Fig 1~~3~~B). For 15 °C grown *Nitzschia sp.* that was measured at 15 °C, the pattern of decrease in effective quantum yield ~~decreasing pattern under P or PAB~~ was similar to that of *Skeletonema sp.*, with lower values under PAB (p<0.001, F=38.8) (Fig 1C, Table A1). In addition, ~~while for~~ PAB exposed ~~cells,~~ *Nitzschia sp.* could only recover to ~50% of the initial value under dim light (Fig 1~~3~~C). However, when 15 °C grown *Nitzschia sp.* were incubated at 25 °C for light exposure, both P and PAB treated cells had higher quantum yields, ~~with less UVR suppression of photosystem~~ PSII ~~compared with 15 °C,~~ and PAB exposed cells ~~could~~ recovered to 75% of the initial value when subsequently incubated under dim light (Fig 1~~3~~D). The increase of temperature (15~~-~~ to 25 °C) and UV radiation also showed interactive effects ~~on~~for both *Skeletonema sp.* (p=0.022, F=2.98) and *Nitzschia sp.* (p=0.046, F=2.5) (Table A2).

The 20 °C grown *Skeletonema sp.*~~, independent of incubation temperatures (20 or 30 °C),~~ showed ~~in~~significant UV inhibition at incubation temperatures of 20°C

(p<0.001, F=8.9) ~~or~~and 30 °C (p=0.033, F=3.1) ~~for most of time points during radiation exposure~~, and recovered more quickly under dim light, especially for the PAB treated cells, compared with samples under 15 °C (Fig 2~~4~~ A, B, Table A1). For *Nitzschia sp.* that were grown at 20 °C, cells showed moderate UV inhibition during radiation exposure (p<0.001, F=10.1), and the quantum yield under PAB treatment only recovered to ~80% at the end of the dim light incubation at 20 °C, while quantum yield recovered to the initial value in cells measured under 30 °C (Fig 2~~4~~ C, D, Table A1). Interactive effects of temperature increase (20~~-~~ to 30 °C) and UV radiation were observed for both *Skeletonema sp.* (p<0.01, F=4.35) and *Nitzschia sp.* (p=0.015, F=3.26) (Table A2).

Skeletonema sp. that was grown and measured at 25 °C showed a similar pattern to that grown under 20 °C during both radiation exposure and subsequent dim light (Fig 3~~5~~A). However, quantum yields decreased significantly once cells were moved into 35 °C, with much lower values observed under the PAB and P treatments (p<0.001) than under 25 °C. However, there was no significant difference between PAB and P treatments under 35 °C (p=0.60, F=0.74) (Table A1). During the dim light period, *Skeletonema sp.* only recovered to ~30% for the P treatment, while there was no recovery after the PAB treatment (Fig 3~~5~~B). For *Nitzschia sp.* measured under 25 or 35 °C, both treatments showed a similar response, with lower values under PAB than under P during the radiation exposure (p<0.001 and F=13.3 at 25 °C, p<0.01 and F=5.4 at 35 °C) (Table A1), while cells could recover to near initial values at the end of the dim light incubation (Fig 3~~5~~ C, D). An i~~I~~nteractive effect of temperature increase (25-35 °C) and UV radiation was only observed for *Skeletonema sp.* (p=0.049, F=2.46) (Table A2).

In the presence of lincomycin, changes in effective quantum yield showed ~~a~~ a ~~similar~~ decreasing pattern ~~along~~ with exposure time for most of the treatments (Figure A3~~2~~-5~~4~~), but with much greater amplitude compared with non-lincomycin treated samples. ~~except for *Skeletonema sp.* incubated under 35 °C, which had relatively lower values compared with samples under 25 °C (Figure A4).~~

The relative UV inhibition ~~induced by UV radiation~~ at the end of radiation exposure is shown in Fig 4~~6~~. Both species ~~had~~ showed the greatest sensitivities under 15 °C, with ~80% and ~70% relative UV inhibition of photochemical quantum yield for *Skeletonema sp.* and *Nitzschia sp.*, respectively. In the range of acclimated temperatures, relative UV inhibition ~~decreasing~~ decreased with increase of temperature for both species. ~~While i~~In the ~~range of~~ short term incubations with a 10 °C increase, UV inhibition of *Skeletonema sp.* was comparable at 25 °C and 30 °C, but increased significantly to ~50% at 35 °C (p<0.01). For *Nitzschia sp.*, relative UV inhibition ~~during short term incubation reached a plateau~~was around 25% ~~,~~ in the temperature range of 25 – 35 °C during the short term incubations~~, of around 25%~~.

During radiation exposure, the repair rates for ~~photosystem~~ PSII in *Skeletonema sp.* varied across~~among~~ the different temperatures, with highest values observed at 25 °C, and lowest values at 35 °C for both radiation treatments (Fig 5~~7~~A). The damage rates gradually decreased from 15 to 25 °C, then increased significantly toward 35 °C (Fig 5~~7~~B) (p<0.001). The ratio of repair rate to damage rate (r : k) showed a unimodal pattern with peak values at 25 °C, and with lowest values under 15 or 35 °C, especially for the PAB treatment (Fig 5~~7~~C).

The repair rate during light exposure for *Nitzschia sp.*, increased significantly in the temperature range of 15 to 25 °C (p<0.001), while kept relatively stable from 25 to 35 °C (Fig 6~~8~~A). The damage rates were quite stable for all temperatures tested, whether cells were acclimated or exposed to short term elevation of temperature, with mean values around 0.075 for PAB and 0.032 for P treatment (Fig 6~~8~~B). The r : k ratio increased with temperature in the range of 15-25 °C, reaching relatively stable values of around 1.50 for P~~AR~~, and around 1.0 for the PAB treatment (Fig 6~~8~~C).

Under dim light, the rate constants for recovery of P~~AR~~-exposed *Skeletonema sp.* were around 0.10-0.15 min^{-1} in the range of 15-30 °C, ~~while~~ but increased significantly to around 0.30 at 35 °C (p<0.01) (Fig 7~~9~~A). The rate constant for recovery of P exposed *Nitzschia sp.* was relatively stable, around 0.25 min^{-1}, ~~in~~ across the range of applied temperature (Fig 7~~9~~B). The rate constant for recovery of PAB exposed *Skeletonema sp.*

showed an increasing pattern from 0.05 to 0.17 min^{-1} in the range of 15-25 °C, but decreased significantly at 30 °C (p<0.05); at 35° values were unable to be estimated due to poor fitting of data points (Fig 79C). No consistent trend was found for the rate constant for recovery of PAB exposed *Nitzschia sp.,* which varied around 0.10-0.15 min^{-1}, ~~in~~across the range of applied temperature (Fig 79D).

Discussion

~~The natural variation of physical and chemical factors, including nutrients, salinity, temperature, light etc., provide major controls that determine the distribution, succession and composition of phytoplankton (Levasseur et al., 1984). In response to these variables, phytoplankton have evolved different strategies of acclimation or adaptation (Irwin et al., 2015;Padfield et al., 2016).~~ In the present~~this~~ study, we found that both benthic and planktonic diatoms were less inhibited by UVR under moderately increased temperature, while the benthic species was more resistant to UVR under the ~~extreme~~ highest temperature applied~~temperature~~, which ~~indicated~~suggests that the tolerance to environmental stress was associated with the niche environment where ~~phytoplankton~~the microalgae are living~~e~~, that would be in turn determine the biogeographic properties of ~~phytoplankton~~the species. These findings imply that temperature is a key factor that mediates the response of diatoms to UVR, while different species have developed distinct mechanisms in response to their particular niche environments (Laviale et al., 2015).

As a basic environmental factor, temperature affects all metabolic pathways, and extreme or sub-optimal conditions are often encountered by various organisms in nature (Mosby and Smith, 2015). The growth response of phytoplankton to temperature varies from species to species, but often shows a unimodal pattern (Brown et al., 2004; Chen, 2015). For the applied temperature range in the present study, the growth rate of the benthic species showed a slight response, while growth increased with temperature to a greater extent in the planktonic species, particularly above 25 ºC. However, life forms in the natural environment are affected by multiple stressors concomitantly (Boyd et al.,

2015). For instance, ~~a~~ recent stud~~y~~ies~~ies~~ have demonstrated that increased temperature would affect phytoplankton interactively ~~affect phytoplankton~~ with light intensity (Edwards et al., 2016), and could alleviate UV direct inhibition ~~on~~ in some sensitive species (Halac et al., 2014). Moreover, in diatoms short-term changes in temperature ~~had~~showed a greater ~~more~~ interaction with UV radiation than did long-term exposure~~with UV radiation in affecting diatoms~~, which ~~was~~was particularly important for intertidal benthic species (Sobrino and Neale, 2007). In the present study, w~~W~~hen species were acclimated under sub-optimal temperature (15 °C), both showed obvious sensitivity to UVR (Fig 1~~3~~). During the recovery period, however, the effective quantum yield of the benthic diatom could rapidly ~~reach~~ regain the highest values within 12 min irrespective of the incubation temperature. The planktonic diatom, however, only performed better under ~~short~~ short-term elevated temperature. This suggests that the benthic species could have broader adaptability ~~in~~ to cope with the highly varied temperature environment they frequently experience (Laviale et al., 2015).

The operation of ~~Photosystem~~ PSII is sensitive to light intensity as well as quality. High levels of PAR~~P~~ and UVR can usually induce significant damage to this complex, while the de novo synthesis of protein can replace the damaged subunit (Aro et al., 1993; Lavaud et al., 2016). The damage rate (k), which represents the efficiency of detrimental effects, showed a different response for the 2 species in this study; in the planktonic species, k was sensitive to temperature change, with the lowest value at the medium temperature, but was quite stable in the ~~–~~benthic species at all temperatures tested. This could be attributed to a decrease in electron transport, or intrinsic differences between benthic and planktonic species~~changes in ultra-structure which resulted in higher intracellular light exposure for planktonic species~~ (Melis, 1999; Nitta et al., 2005)~~–~~, since k of the planktonic *Thalassiosira sp.* also showed sensitivity to temperature change (Sobrino and Neale, 2007). The repair rates (r) and the ratio of r to k further demonstrated that the planktonic species had a relatively lower optimal temperature in response to UVR, with the highest r : k and lowest UV inhibition at 25 °C. In contrast, in the benthic species r and r : k increased steadily and reached relatively stable values

at the highest temperature, and this coincided with lower UV inhibition, implying that although acclimated in laboratory conditions for weeks, this species still had an active mechanism to respond to high temperature and UVR, as might occur in its natural niche environment (Laviale et al., 2015).

In addition to repair processes that are initiated after damage, UV absorbing compounds could directly screen out part of the detrimental radiation, protecting cellular organelles from UV damage (Garcia-Pichel and Castenholz, 1993). In diatoms, however, the spectra of methanol extracts showed only a small absorbance peak in the UVR. Unlike xanthophyll cycle related pigments, UV-absorbing compounds (UVAC) are inducible and only synthesized under long-term UV exposure, indicating that UVAC are not a major protecting mechanism for laboratory cultured diatoms (Helbling et al., 1996). However, the xanthophyll cycle could respond quickly under photo-~~inhibition~~inhibitory conditions, and has been shown to be a major mechanism in diatoms in response to high light or UV (Cartaxana et al., 2013;Zudaire and Roy, 2001). Therefore, the relatively higher absorption in the blue range for benthic species~~,~~ ~~–~~ might indicate that temperature enhances the synthesis of xanthophyll related pigments (Havaux and Tardy, 1996). ~~Therefore,~~ The differences in absorption spectra of extracted pigments suggest~~ed~~s that to better understand the spectral ~~-~~-dependent responses to UV radiation, biological weighting functions should be introduced in this kind of work (Neale et al., 2014).

The temperature dependent response to UVR has major implications for phytoplankton. With the continuing emission of greenhouse gases, the surface seawater temperature is predicted to increase by up to 4 °C by the end of this century (New et al., 2011), and this could potentially re-shape the phytoplankton assemblages (Thomas et al., 2012). While the situation might be more complex in the natural environment with the consideration of interaction of UVR with other factors (Beardall et al., 2009), for unicellular green algae, an increase of temperature could mitigate UVR harm for temperate species, while exacerbating UV inhibition for polar species (Wong et al., 2015). Moreover, the tolerance of phytoplankton to extreme temperature would be

latitude dependent; for tropical areas where the temperature is already high, an increase of temperature reduced the richness of phytoplankton (Thomas et al., 2012).

The present study showed a differential response to UV radiation for two diatoms from contrasting niches. As predicted, the benthic species had a higher tolerance to the combination of extreme temperature and UV radiation, which can be attributed to the environment in which were living. Below the optimal temperature, both species performed better in response to UV radiation under elevated temperature, suggesting that the natural variation of temperature due to changes in the heat flux from the sun or meteorological events would alter the extent of UV effects on primary producers, and therefore the aquatic ecosystem (Häder et al., 2011). Furthermore, considering the projected global warming scenarios, UV radiation could impose different impacts on phytoplankton with respect to the regional differences (Beardall et al., 2009; Xie et al., 2010).

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Fig legends:

Fig 1 The quantum yields of 15 °C grown *Skeletonema sp.* and *Nitzschia sp.* under P or P+UVR for 1 hour exposure and subsequent recovery under dim light (gray area) for 1 hour, that were incubated and measured at 15 °C (A: *Skeletonema sp.*, C: *Nitzschia sp.*) or 25 °C (B: *Skeletonema sp.*, D: *Nitzschia sp.*)~~.~~, vertical lines represent SD, n=3.

Fig 2 The quantum yields of 20 °C grown *Skeletonema sp.* and *Nitzschia sp.* under P or P+UVR for 1 hour exposure and subsequent recovery under dim light (gray area) for 1 hour, that were incubated and measured at 20 °C (A: *Skeletonema sp.*, C: *Nitzschia sp.*) or 30 °C (B: *Skeletonema sp.*, D: *Nitzschia sp.*) , vertical lines represent SD, n=3.

Fig 3 The quantum yields of 25 °C grown *Skeletonema sp.* and *Nitzschia sp.* under P or P+UVR for 1 hour exposure and subsequent recovery under dim light (gray area) for 1 hour, that were incubated and measured at 25 °C (A: *Skeletonema sp.*, C: *Nitzschia sp.*) or 35 °C (B: *Skeletonema sp.*, D: *Nitzschia sp.*) , vertical lines represent SD, n=3.

Fig 4 The relative UV inhibition ~~induced by UVR~~ on the photosystem II of *Skeletonema sp.* (A) and *Nitzschia sp.* (B) under grown or short term elevated temperature, vertical lines represent variance~~SD, n=3~~..

Fig 5 The repair rate (A) and damage rate (B) of photosystem II in *Skeletonema sp.* during P or P+UVR exposure under grown temperature (acclimated) or short term elevated temperature (short_term), and the ratio of repair to damage rate (C), vertical lines in panel A and B represent SD, n=3, while vertical lines in panel C represent variance. Data points with different lower case letters (blue for P treatment, and red for PAB treatment) indicate~~d~~ significant differences among temperature treatments.

Fig 6 The repair rate (A) and damage rate (B) of photosystem II in *Nitzschia sp.* during P or P+UVR exposure under grown temperature (acclimated) or short term elevated temperature (short_term), and the ratio of repair to damage rate (C), vertical lines in panel A and B represent SD, n=3, while vertical lines in panel C represent variance. Data points with different lowercase letters (blue for P treatment, and red for PAB treatment) indicated significant differences among temperature treatments.

Fig 7 The rate constants for recovery of P exposed *Skeletonema sp.* (A) and *Nitzschia sp.* (B), and rate constants for recovery of PAB exposed *Skeletonema sp.* (C) and *Nitzschia sp.* (D) under dim light, samples were incubated under grown temperature (acclimated) or short term elevated temperature (short_term), vertical lines represent SD, n=3. Data points with different lowercase letters (blue for P treatment, and red for PAB treatment) indicated significant differences among temperature treatments.

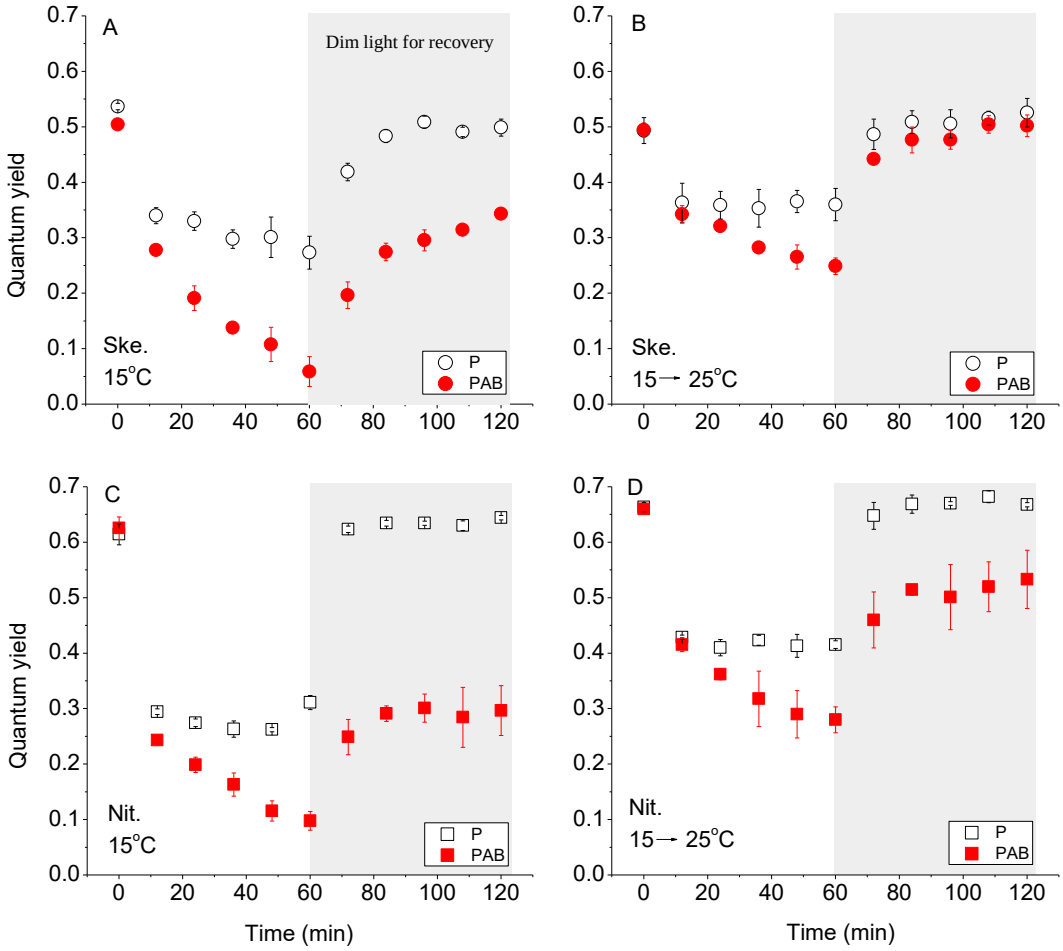

A
Dim light for recovery
Quantum yield
Ske.
15°C
P
PAB
B
Ske.
15→25°C
C
Nit.
15°C
D
Nit.
15→25°C
Time (min)

Fig 1

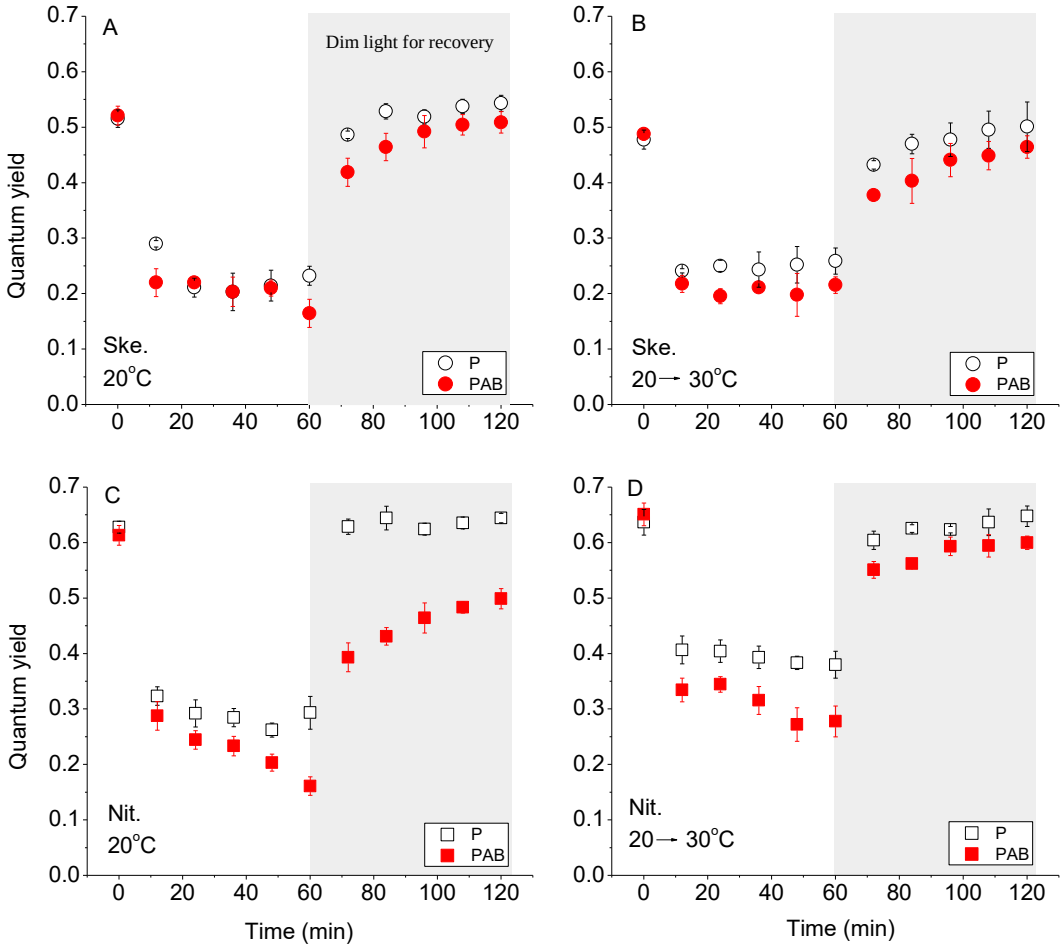

A
Dim light for recovery
Quantum yield
Ske.
20°C
P
PAB
B
Ske.
20→ 30°C
C
Nit.
20°C
D
Nit.
20→ 30°C
Time (min)

Fig 2

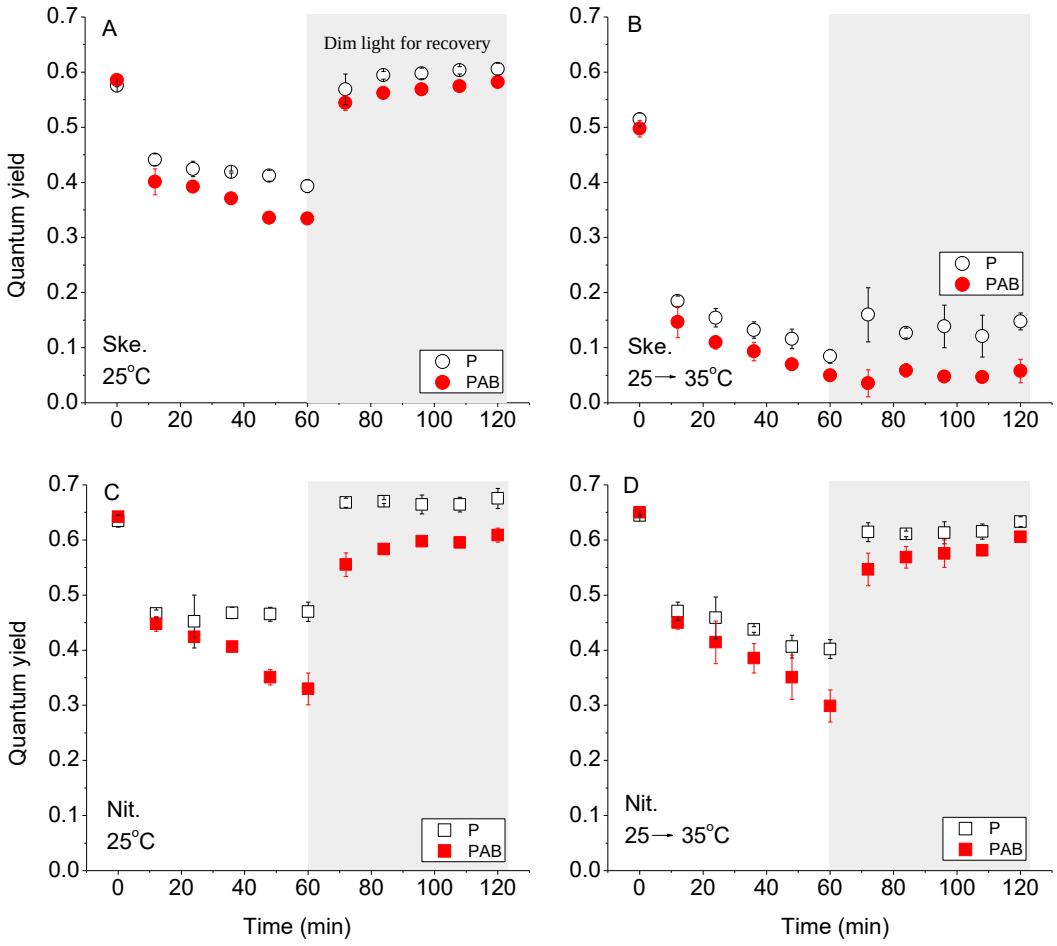
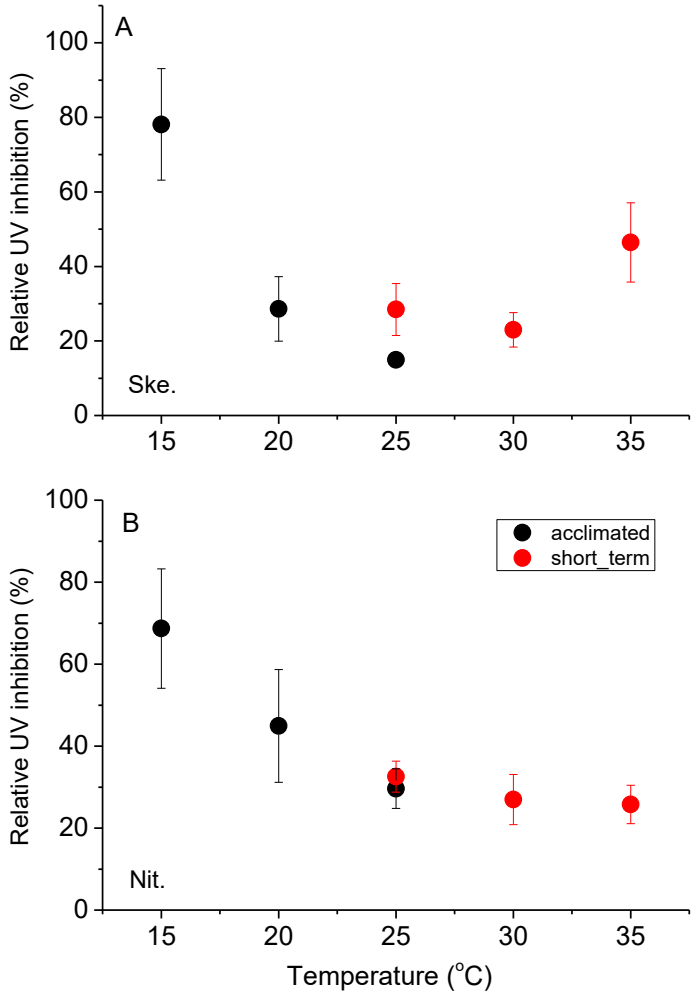
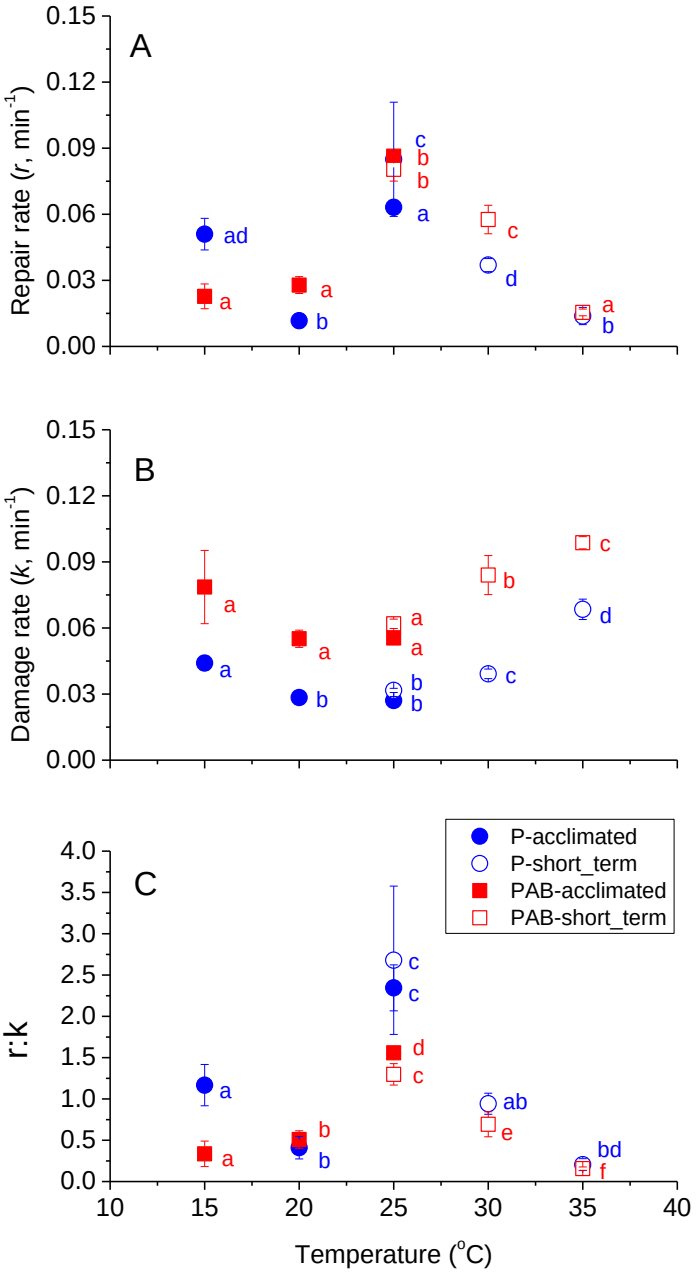

A
Dim light for recovery
Quantum yield
Ske.
25°C
P
PAB
B
Ske.
25→ 35°C
C
Nit.
25°C
D
Nit.
25→ 35°C
Time (min)
Time (min)

Fig 3



622
623
624 Fig 4



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632 Fig 5

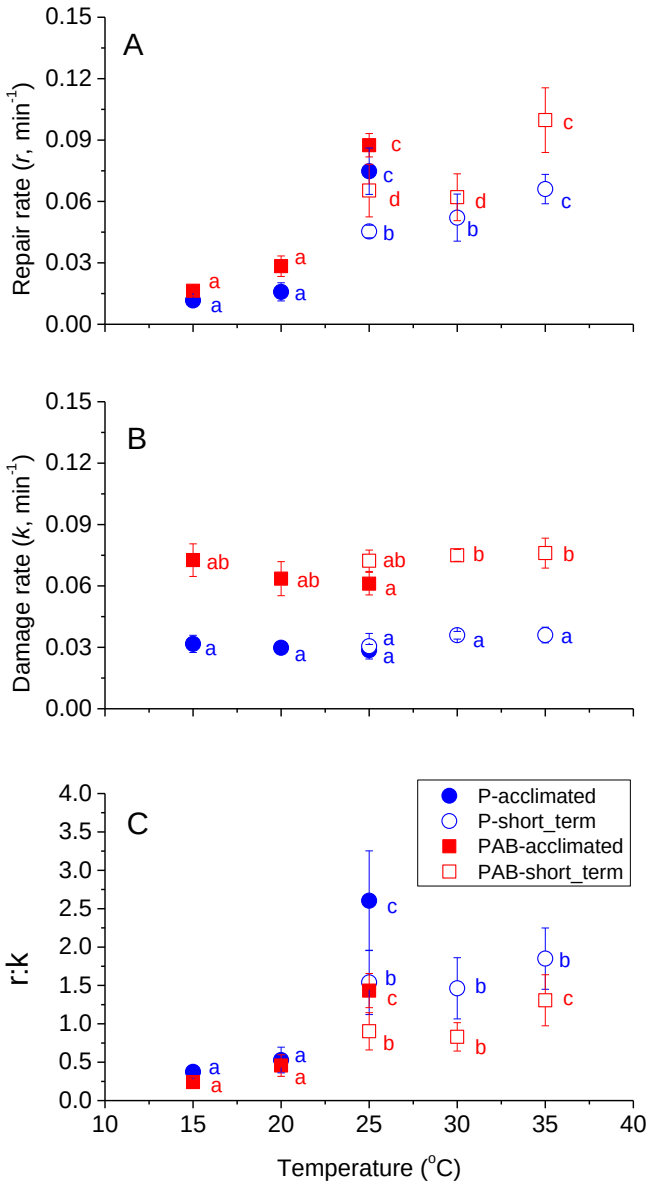
A
Repair rate (r, min-1)
0.15
0.12
0.09
0.06
0.03
0.00
B
Damage rate (k, min-1)
C
r:k
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
10
15
20
25
30
35
40
Temperature (°C)
P-acclimated
P-short_term
PAB-acclimated
PAB-short_term

Fig 6

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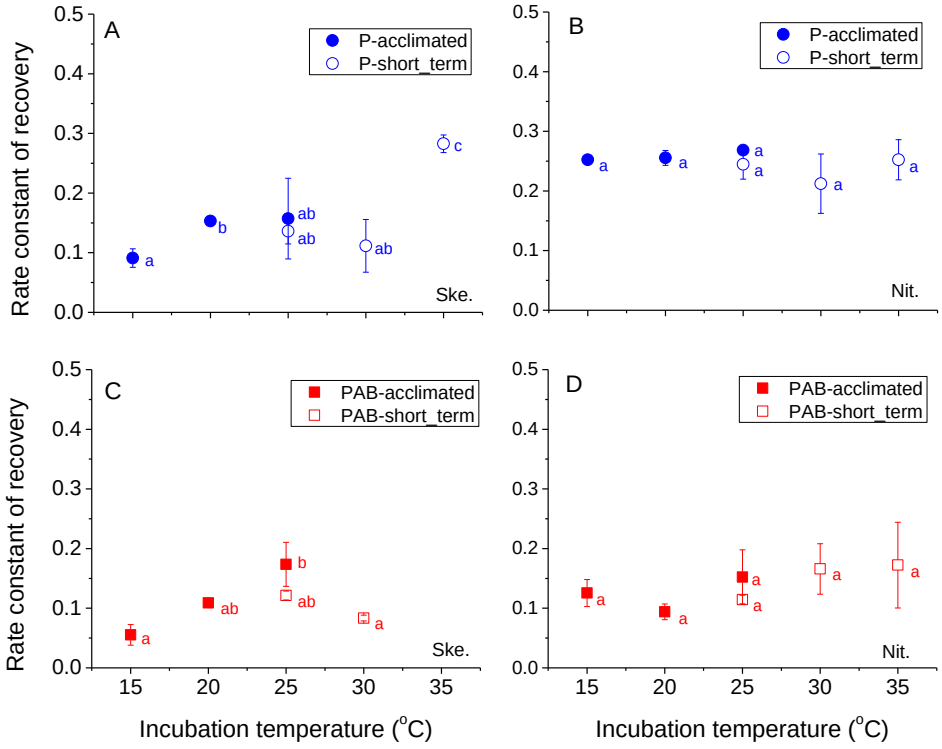

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643 Fig 7

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Supplementary:

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

655

656 Table A2 The statistical results of RM-ANOVA for effective quantum yields during
657 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

658

Table A3 R square values for curve fitting with 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
Nitzschia sp	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

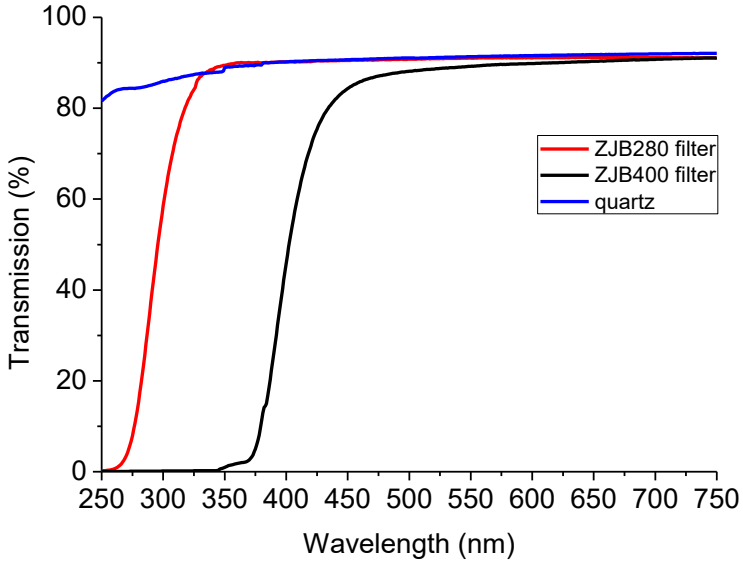

Fig A1The transmission spectra (in percentage) of different cut-off filters (ZJB280, ZJB400) and the quartz tube between 280 and 750 nm.

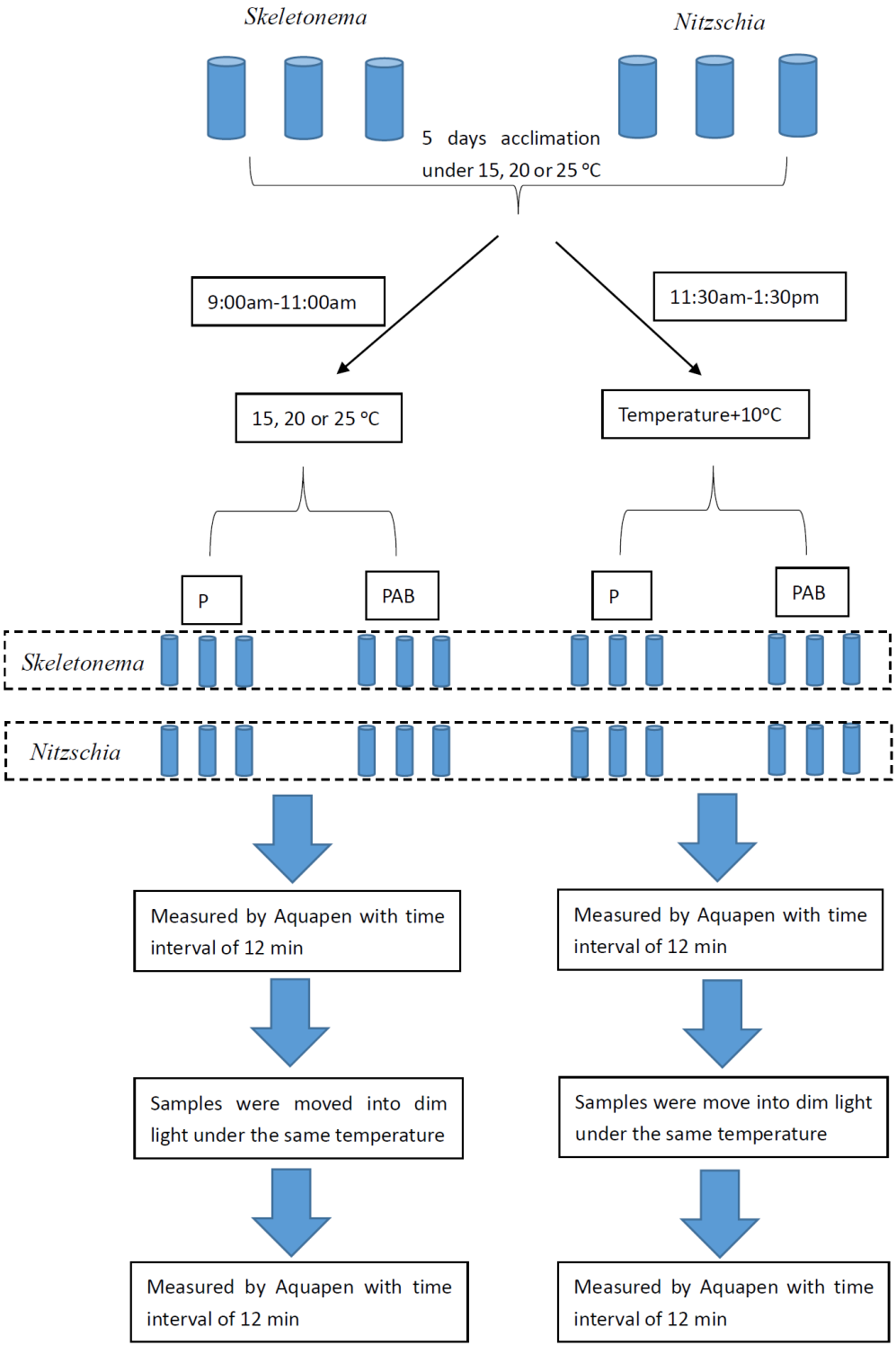

Fig A2 The illustration of the experimental design from culturing to light exposure experiments.

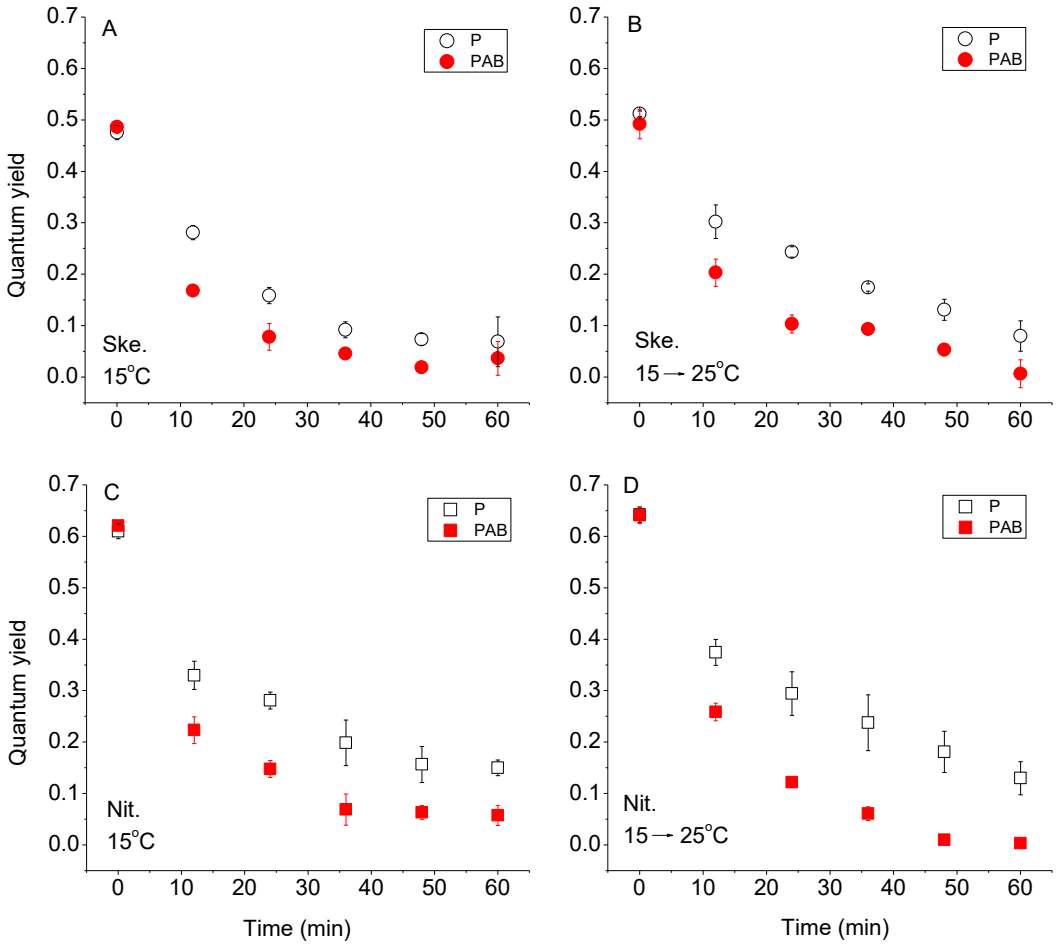

Fig A3 The quantum yields of 15 °C grown *Skeletonema sp.* and *Nitzschia sp.* under P or P+UVR for 1 hour exposure in the presence of lincomycin, that were incubated and measured at 15 °C (A, C) or 25 °C (B, D) , vertical lines represent SD, n=3.

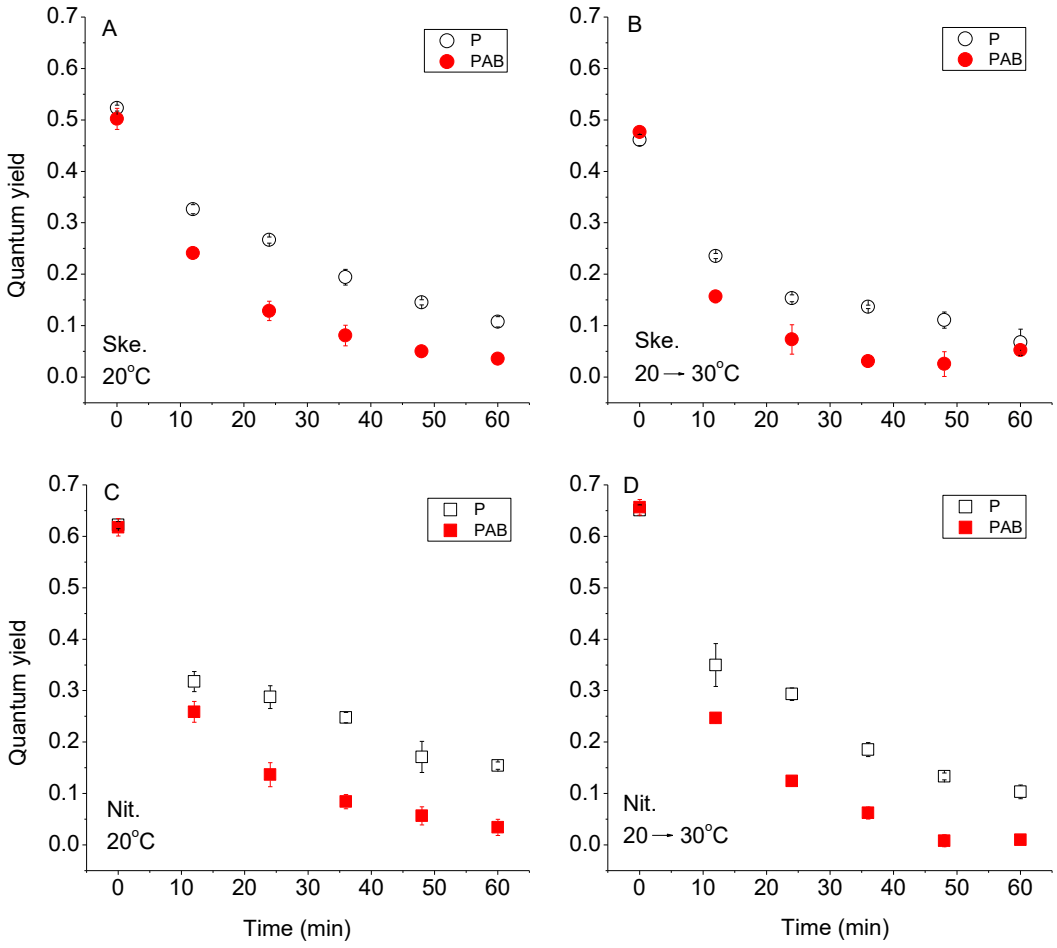

Fig A4 The quantum yields of 20 °C grown *Skeletonema sp.* and *Nitzschia sp.* under P or P+UVR for 1 hour exposure in the presence of lincomycin, that were incubated and measured at 20 °C (A, C) or 30 °C (B, D) , vertical lines represent SD, n=3.

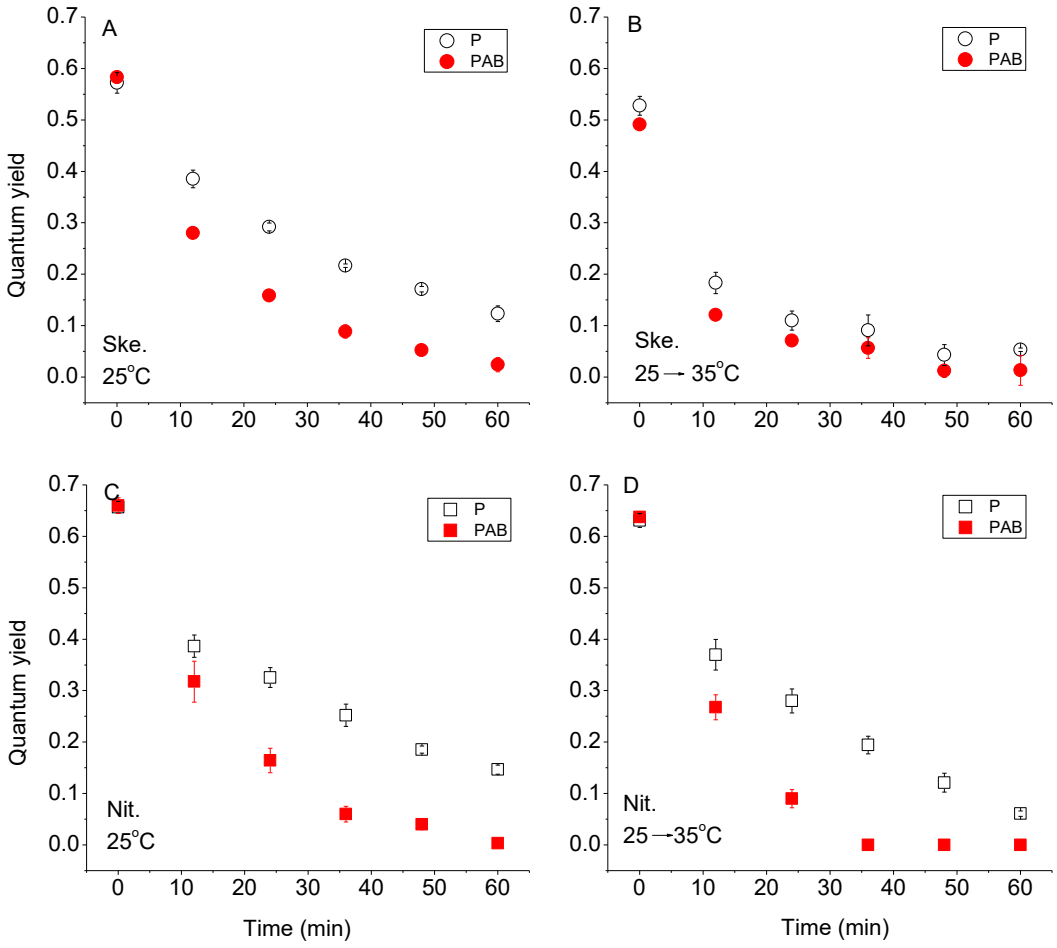

Fig A5 The quantum yields of 25 °C grown *Skeletonema sp.* and *Nitzschia sp.* under P or P+UVR for 1 hour exposure in the presence of lincomycin, that were incubated and measured at 25 °C (A, C) or 35 °C (B, D) , vertical lines represent SD, n=3.

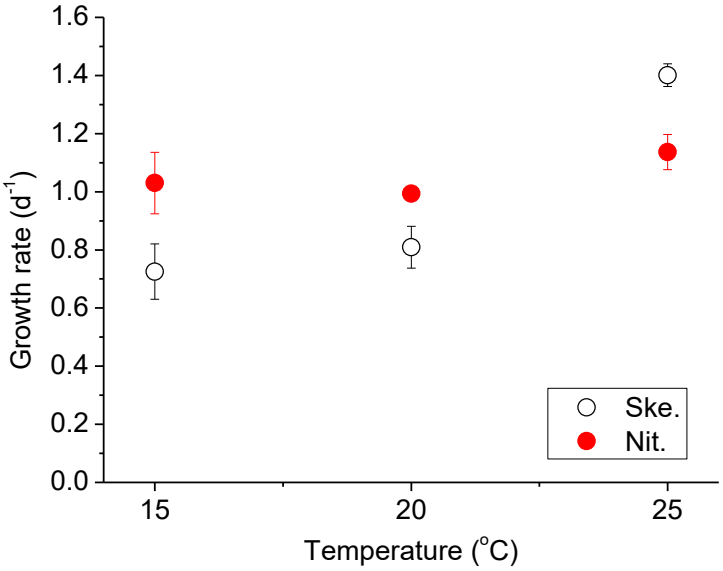

Fig A6 The specific growth rates of both species under different temperature levels, vertical lines represent SD, n=3.

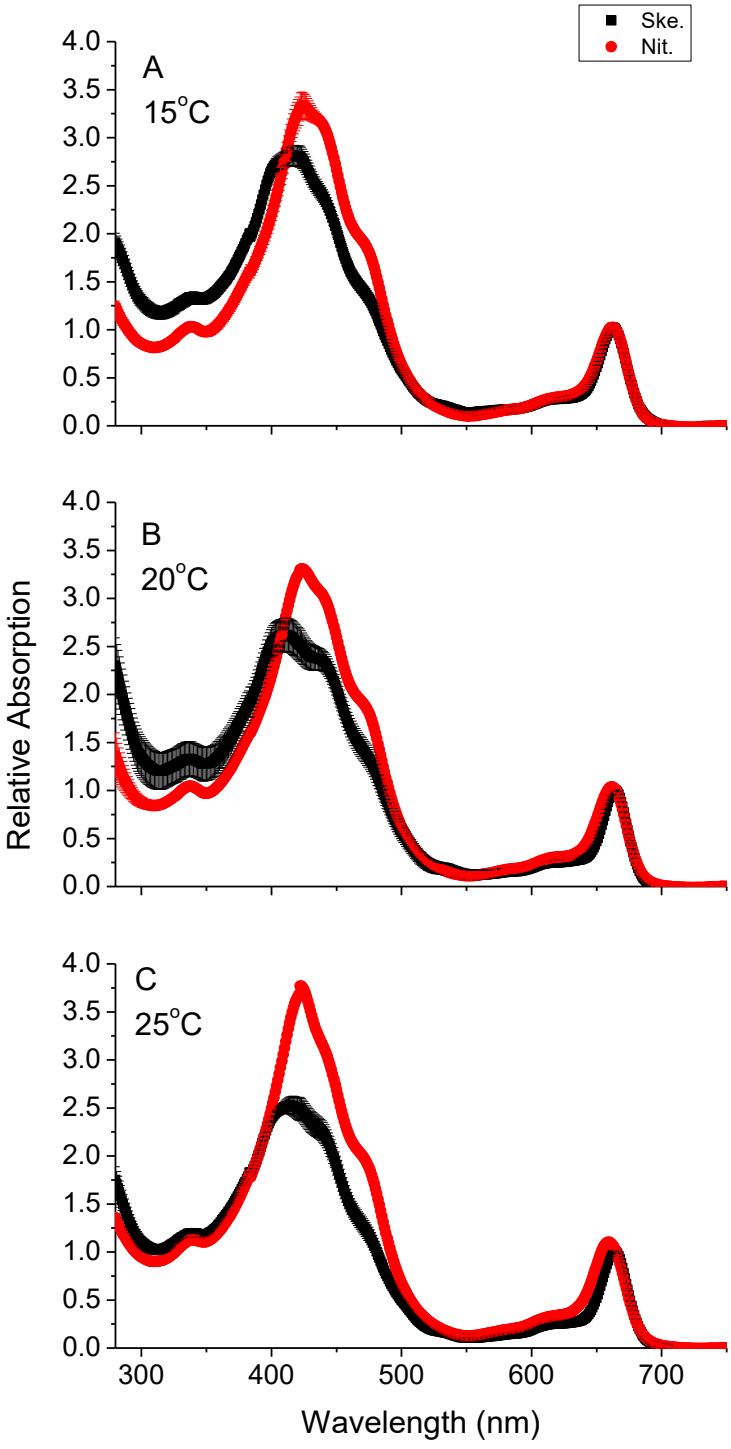

Fig A7 The absorption spectra of methanol extracts of *Skeletonema sp*. and *Nitzschia sp*. cultured under different temperature, spectra were normalized with value set as 1.0 at wavelength of 665nm, vertical lines represent SD, n=3.