**Responses to reviewer comments on:**
**Photophysiological state of natural phytoplankton communities in the South China Sea and Sulu Sea by Cheah et al.**

We thank the referees for their valuable comments which help to improve our manuscript. Please find our response to referee's comments in "**blue**" and changes in the revised manuscript highlighted in "red".

**Short comment by Dr. T. Smyth**
This paper presents a comprehensive set of measurements (physical, chemical and biological - although the latter inferred by HPLC techniques in general) in the South China Sea and Sulu Sea. The authors look at the different drivers behind the different physiological stresses on the phytoplankton in these regional seas. In terms of description of the area and its attendant hydrography, nutrient stresses and the like it is a useful contribution to the field: indeed I think there are few studies of this particular area that I am aware of. However, on p12118 and line 4 the authors state that: "... the exclusion of photoadaption information in the model resulted in a 35% underestimation of primary production in the northern SCS." I think that what is missing from this paper is a wider application of the results to modelling studies of the area. This would then give the reader more of a feel for "what have we learnt from this study" rather than a simple presentation of results with only an attempt statistically (in univariate space) to determine correlation and "drivers". This might be an unrealistic criticism as the physical oceanography and biogeochemistry are highly complex in this region and there may be little or no ecosystem model skills amongst the authors. If the authors are to develop this work further beyond the (albeit good) descriptive stage, this is where I would suggest they take things.
**We thank Dr. Smyth for his comments and agreed that a modeling experiment based on the results obtained from this study could help to elucidate the implication of photophysiology. However, this is beyond our experimental focus of our study, but now also according to the suggestions of the referees, we more carefully analysed the photoadaptation and photoacclimation of phytoplankton in these regions and used this understanding to predict the implications in the future for these regions under a changed environmental condition.**

**Referee #1**
This MS examines phytoplankton photophysiological variability along an environmental gradient in the South China and Sulu Seas. Overall, the idea behind the MS is sound – this is not a well-studied region and currently efforts to model primary productivity for this region are confounded by a lack of understanding of the underlying photobiology. Despite having a great data set, the MS suffers from a lack of direction and it's impossible to know what the readers are supposed to take away. Primarily, the MS lacks clear (overall as well as specific) aims/objectives/hypotheses; therefore, it is difficult to follow what the authors are really "looking for" through their data analysis, which in itself suffers form a lack of depth (and is presented/examined in a very 1-dimensional manner). I was frustrated and disappointed as I read on through the MS since this is a very nice data set that could really be used to explore something more novel and elegant. There is definitely a paper in here somewhere but it needs a major re-think. At present, I cannot recommend that the MS is published but should be resubmitted as a new MS for re- review. Most of my comments at this stage reflect what the authors perhaps need to (re) consider in getting

much more out of the data they have and build on recent papers that have moved forward our understanding as to how the environment regulates phytoplankton communities and (photo)physiology.

General Comments

1. It is not clear just what the aim of the MS is. It reads as a data paper, which is fine given this is not a well studied region (but is it enough for the impact factor of Biogeosciences?) but it really comes across as confusing in what the authors are attempting to do by describing the patterns. Are the trends they observe expected and consistent with what one would expect for the environmental gradients encountered? – similar type studies have been published from other seas/oceans, including complex environmental gradients, that should perhaps be used to better inform the study up front, e.g. hypothesis formulation. The authors appear to have an interesting (unique?) oceanographic environmental setting and should draw more on this. As such, the introduction needs to be more detailed and well-thought through to inform specific aims aims.

**As recommended, we have rewritten the Introduction section according to reviewer's comments and believe the Introduction now contains a clearer objective and hypothesis. Explicitly, we now added a paragraph (see 2nd last paragraph in the Introduction) on the benefit of photophysiological studies to enlighten the competency of phytoplankton. We also added multivariate statistics to the previous univariate statistics (see Sections 2.7 and 3.5) and changed the previous 1-dimensional Figures 2 and 4, as suggested, to maps. These new results support our discussion (see Section 3.5).**

2. The MS is undermined by two major problems associated with the Figures/data analysis (i) The quality of the figures makes them very difficult to examine critically and pull out the key trends within and across the environmental grandient(s); (ii) the data is analysed in a very 1-dimensional manner such that it's impossible to see the trends and patterns. I'm really surprised that the authors don't try to elaborate on some of the more elegant approaches used previously e.g. Moore et al. 2005 DSR that do a really nice job of linking the environment and the photophysiology across environmental gradients. As presented, this does not feel as though the MS (ands the unique data set for this region) is really moving the field forward. On this note, the data analysis really needs a re-think. Much of the analysis is univariate and the only attempt to quantify patterns is restricted to a series of correlations (Table 3) that provides no insight as to how the complex multivariate environment is operating to regulate the taxonomy over the physiology. The authors really need to take a more multidimensional space type approach; either relatively 'simplistically' such as that used by Moore et al. (2005) (whereby values for parameters are visualised in multidimensional space – I appreciate the authors are trying to do this for the cast data via the contour plots but these do not really convey the complexity and so more treatment of the data (binning) is really needed to extract the key points) – or a more complete multivariate statistical analysis. I'm really surprised the data analysis has not been taken to this level to help streamline the complexity of the issues at play. The key trends/messages are lost but again I feel this reflects that the lack of clear messages upfront confounds just how the data needs to be treated.

**As pointed out above, we have modified the surface plots (Fig.2 and Fig. 4) and have analysed the data in more dimensions.**

Specific Comments

PG12116LN5. The abstract states that "This study investigates the photophysiological state of natural phytoplankton communities" but it needs to clearly describe why this is important to do.

**We have modified the second sentence of the abstract to: "However, still little is known about the photophysiological state of natural phytoplankton communities which is required to predict the influences of anthropogenic changes (e.g. pollution, sea temperature rise) on phytoplankton in these oceanic regions."**

PG12118LN3. The authors begin to distil to the main issue in the introduction by stating that little "photoadaptation information" exists with which to inform models but this train of logic falls short since it is not clear (i) what is this information specifically and (ii) how the study here has been designed (i.e. the set of measurements used) to address this limitation.

**We have clarified this issue in the revised Section 3.4 in which we combined the information gained from our previous Sections 3.4.2, 3.5 to 3.6 to identify the characteristics and time scales of phytoplankton response to light.**

PG12119LN3. I was intrigued by the idea of sampling from a moon pool. What is the residence time of water here and is there any delay between where the ship is sampling and the water that is comprising the moon pool. Later in this paragrah I did not understand why samples for only pigments and absorption were taken from these shallow sites. In fact, it's entirely unclear the (mis)match between when the 'standard' oceanographic and FRRf measurements wre made and when absorption/HPLC samples were taken – this needs to be better explained and when (and when not) there are matched samples available.

**The moon pool on RV Sonne is an opening at the base of the hull. For our surface sampling, seawaters were collected using a peristaltic pump in which a clean silicon tube connected to the peristaltic pump was lowered to the bottom of the moon pool (~6m), close to the base of the hull. Water sampling was always completed within 5 minutes. Hence, there should not be an issue with residence time. The moon pool provides a platform in which seawater sampling for various scientific studies can be carried out simultaneously using the appropriate pump for each study. In our sampling, the peristaltic pump operated at low pressure reduced the risks of damaging phytoplankton cells during sampling as indicated in microscopy in which the sampled cells were still intact. Also the FRRf measurements were taken from the moonpool and the FRRf values have been averaged over 10 min intervals which incorporate the sampling time of water samples at each collocation. The physical parameters of surface stations were taken from the thermosalinograph which samples water from the same depth as the moonpool. These values were also averaged over 10 min. For our further analysis, these measurements are as well direct collocations to the water sample measurements.**

**Sampling for flow cytometry and nutrient analyses was also carried out at local boat stations.**

**CTD casts were deployed at every CTD stations, whereas FRRf casts were carried out at every CTD stations except for Station 2 and 8. Sampling for pigments, absorption, flow cytometry, microscopy, and nutrient analyses were carried out every at CTD station except for Station 8.**

**We have included all the information mentioned above in Section 2.1.**

PG12121LN1. I appreciate that the detail associated with the relationship between TChla and Fm and dealt with in section 2.6 but it would be good to see the resulting trends (or at least report the regressions/correlation details and how well they hold across sites once NPQ is accounted for); on this note also, why use Fm and not F0 or even Fv? There are various arguments behind how well each of these parameters can/should relate to biomass and a well reported issue and the authors should perhaps explore this in a little more detail to identify the most robust approach based on their fluorescence data.

**We have included number of collocations, regression and uncertainty details on the end of Section 2.6. The reason Fm was selected over F0 to derived TChla is because F0 represents the state of maximum quenching and is very difficult to be measured accurately. In contrast, Fm represents the state in which all PSII reaction centers have been reduced and can be measured more accurately. One of the reviewers of our previous paper (please see http://www.biogeosciences-discuss.net/8/7165/2011/bgd-8-7165-2011-discussion.html) has recommended using FRRf-Fm over F0 for deriving continuous TChla data set. Nevertheless, we also tested to derive TChla from F0 in the same way by calibrating them with the collocated HPLC data and the results were very similar to TChla inferred from Fm.**

PG12122LN1. There's some issues with the FRRf approach that need to be clarified: (i) what was the processing software used to derive the fluorescence parameters (if the default Chelsea software then this is known to be erroneous – see papers by Sam Laney – and in which case the error here again will need to be sensitised); (ii) The approach to yield qP by Suggett et al. 2006 is actually the ratio of the PSII yield from the light and dark chambers – this is the most robust approach to yield qP from FRRf deployments in situ since F0' cannot be strictly determined. The authors need to be more clear as to what they have done here; (iii) the prime for the fluorescnec parameters determined under actinic light should follow the fluorescence parameter and not be embedded halfway through e.g. sPSII' not s'PSII (or Fm' not F'm) as they currently read; (iv) blanks seem logical but need a reference perhaps to support the in situ blanking approach (perhaps Smythe et al 2004?) – even so, it would be good to know what typical % of the signal was represented by the blank (i.e. how significant it was).

**(i) Our FRRf data were previously analysed using Chelsea software. We are grateful for this suggestion and have reanalysed all our FRRf data based on Sam Laney's v6 MATLAB code. We have included this information in Section 2.4. We thank Sam Laney for his generosity in sharing his MATLAB code and have acknowledged him in the Acknowledgement section.**

**(ii) The qP was derived as in Suggett et al. (2006) and Raateoja et al. (2009). Although F0' cannot be strictly measured during the profiling mode, the ~1s transient dark adaptation provided by FRRf dark chamber under ambient irradiance were**

**measuring F0 at ambient irradiance at the condition where 0<qN<1 and qP close to 1. This assumption allows the determination of qP based on FRRf- Fq' (light chamber)/Fv' (dark chamber) and is described in details in Raateoja et al. (2009) and Suggett et al. (2010). We have included this information in Section 2.4.**

**(iii) All fluorescence parameters under actinic light have been renamed as suggested.**

**(iv) Background fluorescence was between 2% to 13% with no clear pattern at the surface or in vertical profiles. We have included this information in Section 2.4.**

I did not follow sections 3.3. and 3..4.1 (and to some extent 3.4.2) whereby there are separate sections on phytoplankton community (obtained from diagnostic pigments) and then on the changes of the major pigments. Seems like the same issue is really being considered at least twice and there could be much better integration here to state how the major pigments changes and how this relates to (i) changing community versus (ii) changing physiology.

**Section 3.3 and 3.4 have been incorporated into a single section (3.3) with pigment profiles being described before CHEMTAX-derived community structure. We have also swapped Fig 5 and Fig 6.**

**We have also included the information on pigment changes vs physiology in Section 3.4.**

Section 3.4.2. in terms of "acclimation" makes sense but is almost impossible to tease out the influence of taxonomy issue – it may make more sense to simply define what are the photosynthetic versus non-photosynthetic pigments and how these rations are changing? Again, a more transparent analysis of these features in relationship to the environment would really help. At this stage, why not use this pattern of logic to better link the taxonomy to the physiology. Suggett et al use a neat approach in their 2009 paper (and sure the authors attempt to build on this through their Fig. 10 but it is currently ineffective since depth may not be the key variable required to interpret the variability – perhaps look at diagnostic pigments of taxa versus acclimation here instead?). The authors can bin this data in all sorts of ways depending on the main questions being asked of the data and so much more could be done here.

**We have modified Section 3.4.2 (now in Section 3.4) by discussing response of phytoplankton to light looking at the different information from pigment, absorption and fluorescence data. We also included a statistical analysis between surface PAR and photoprotective carotenoids. We finalised the section by discussing the time scales of the response and driving main conclusions on photoadaptation versus photoacclimation of the phytoplankton in our studied regions (shallow versus deep ocean stations, surface layer versus SCM layer).**

On a similar note, section 3.6. currently only superficially considers whether qP or sPSII (i.e. NPQ) are driving how the cells are primarily responding to environmental change but again so much more could be done to examine whether the two co-vary (but normalised in some way) or whether one is much more 'plastic' than the other; similarly, how these actually vary relative to the changing light environment across environments. The authors also have the data on the xanthophyll pigments so why not analyse these in relation to the fluorescence parameters rather than speculating that they may be involved?! This is yet another example that I feel the authors

still have a lot to do with this data set to not only do it (and their hard work in collecting the data) real justice but also highlight more transparently what are the novel messages here. Later sections (e.g. photoacclimation) are just too superficial without the authors really getting their teeth into the detail earlier.

**As suggested, we have included this information in Section 3.4 showing and discussing the results of PC (photosynthetic carotenoids) versus TChla and NPC (photoprotective carotenoids) versus TChla in correspondence to the fluorescence and phytoplankton absorption data.**

Reviewer #2

The manuscript presents Chl-a,phytoplankton pigment, light absorption and photophysiological parameter data along a transect in the South China Sea and Sulu Sea. These properties are compared to environmental gradients (temperature, salinity, macronutrients, light and wind). The paper is generally well written and the data are clearly presented. However, the analysis and interpretation of the data is rather weak. I didn't feel that anything new was learnt beyond reporting the data. Although the data are certainly of interest, I would question whether the manuscript is insightful enough for publication in Biogeosciences in it's current form. I recommend either a more rigorous interrogation of the data and/or better use of the literature to provide stronger evidence of what is learnt in the current study. I have included some general and specific comments that I hope the authors will find useful in a revision.

General comments:

The description of the data seems quite labored and some figures seem unnecessary. For example: - The dissolved oxygen data does not seem to contribute to the main focus of the manuscript (which is photophysiology). If it is not needed for the story, I suggest removing it. - Are both the absolute (Fig 6) and chl-a normalized pigment concentrations (Fig 7a,b,c,d) really needed? I also didn't see the need for both Fig 10A-F and Fig10G-L, which illustrate the same data? - Perhaps it would help to have a clearer objective or hypothesis, so that is more obvious what the data are being used for.

**As suggested, we have removed oxygen data from the manuscript. We have also modified Section 3.4.2 (now Section 3.4) and Fig 7.**

Interpretation of the data was mainly via a correlation analysis, but unfortunately I did not feel that much was learned by this approach. Co-variability in photophysiological properties and environmental variables, such as T, S, nutrients, could be seen quite clearly in the figures. Whether or not the correlations are significant seems, to me, rather arbitrary. Also: - The main drivers of photophysiological properties were light and nutrients (P12135 L1-6) but light was not part of the statistical analysis. - Be care- ful not to assume causation from correlation. For example, in Section 3.8: "These results indicate that an increase in Si supplied by river outflows (stations close to the coasts at Kuching, Kota Kinabalu and northeasat of Sabah) contributed to higher con- centrations of diatoms and cyanobacteria (Fig. 4c), in which affecting the abundance of Prochlorococcus at the surface." Why would Si availability be important to cyanobacte- ria or Prochlorococcus? - Some of the interpretation of the stats seems contradictory, e.g.: P12134 L11: "significant correlations between Si and Fuco . . ., Si and Zea . . ., and a negative correlation between Si and DVChl a were observed at the surface" P12134 L 19: "there is no significant

correlation between Si and major phytoplankton pigments".
**We have now discussed in more depth the drivers of phytoplankton growth in the new Section 3.5: Explicitly, we added multivariate statistics to the previous univariate statistics (see Sections 2.7 and 3.5) and changed the previous 1-dimensional Figures 2 and 4 to maps.**

Specific comments:
P12119 L4: Were there any contamination issues when collecting nutrient samples from a moonpool? (Can you provide some resistance in the text?). Also, please state the limit of detection for nutrient measurements.
**The moon pool on RV Sonne is an opening at the base of the hull. Seawaters were collected from moon pool via a clean tube connected to a pump. The clean tube was lowered to bottom of the moon pool (~6m), close to base of the hull. Hence, the sampled waters were "fresh" and not the one trapped in the moon pool. Care was taken during sampling and measurements plus nutrients were determined based on triplicate samples. Based on the precision of nutrient results, we believe the nutrients values are reliable. We also added the detection limit for the nutrient measurements. This information was added to Sections 2.1 and 2.2.**

P12119 L19: Please include some information about calibration of the CTD sensors (incl. oxygen).
**Calibration information of CTD sensors has been added to the 2nd paragraph of section 2.1.**

P12121 L1-25: I found this sections hard to understand. Can it be simplified? - I suggest removing "a total of 10 PAR profiles were measured at 9 stations. During the cruise, Station 1 and 3 were very shallow to determine zeu which was confirmed by both zeu and zeuPAR. For Station 5, PAR profiles were measured at two daytime casts while TChl a measurements were only available on a third cast. Hence, the compar- isons between zeuPAR and zeu for Station 5 were carried out based on the average of the PAR profiles (68.05 m; the two cast were only 1 m apart). So finally" So that the sentence reads: "The zeu values were then validated with the collocated zeuPAR values. Out of 14 CTD stations, seven stations with collocated zeu and zeuPAR could be compared in order to verify the zeu values..."
**As suggested, we modified this section.**

P12122 L17-21: What were the implications of only collecting FRRFf blanks in the surface mixed layer and chlorophyll maximum? Please provide some assurance that this simplification is adequate? i.e. how big were the blanks, how much did they vary compared to the unfiltered samples and were they representative of the assumed layers?
**The reason blank measurements were only carried out at the surface and chlorophyll maximum layer is because background fluorescence was mostly from CDOM, which is usually high at the surface and chlorophyll maximum layer (Suggett et al., 2005). Background fluorescence were between 2% to 13% with no clear pattern at the surface or in vertical profiles. We have included this information in the end of Section 2.4.**

P12122 L26: 13cm and 25cm Whatman GF/F filters seems rather large for HPLC and, particularly, phytoplankton light absorption measurements (where analysis is normally conducted on in tact filters)? Are these sizes correct?
**The filter sizes were actually 25mm and 47 mm for HPLC and absorption, respectively. We are grateful for this comment and have corrected this accordingly.**

P12123 L7: The phytoplankton community structure information is entirely dependent on a CHEMTAX model. Were the modeled phytoplankton community structures ground trothed in any way? If not, please provide additional reassurance that the CHEMTAX starting points from Zhai et al. 2011, which were for winter conditions, were reliable for this application.
**We have cross-checked our CHEMTAX results with representative microscopy data and flow cytometric data (details on methods in Section 2.5) and observed good agreement between those data sets. We believe therefore that the matrix from Zhai et al., 2011 is applicable in our study. Details can be found in the revised Section 3.3.**

P12126 L10-L28: Please be specific when using the terms "TChla" and "biomass" when describing the HPLC –derived phytoplankton community structure. TChla is initially defined as "total chlorophyll-a" but is this really what you mean when talking about the Chl-a contributions of the different phytoplankton? Also, please avoid using the term biomass when you really mean Chl-a. It would be helpful to state the property in the unit (i.e. mg Chl-a m-3 rather than mg m-3) to avoid confusion. In particular, please specify whether you are talking about chlorophyll (mg Chl-a m-3) or carbon biomass (mg C m-3) in Fig 5.
**We have replaced the term "biomass" with "TChla" and changed the unit mg m-3 to mg Chl a m-3. The units in Fig. 5 (now Fig. 6) have been corrected.**

Section 3.4 and 3.3. I suggest swapping these sections around (and also swap Fig 5 and 6). It makes more sense to describe pigment concentrations first because those are actual measurements, while community structure is a derived quantity.
**Section 3.3 and 3.4 have been modified. In section 3.3, we first describe the pigment composition and then discuss the CHEMTAX-derived community structure which is shown to be reflected in microscopic and flow-cytometric data. We have also swapped Fig 5 and Fig 6.**

P12127 L7-L16: I suggest moving the sentence "A linear regression of r2 = 0.89 ($p < 0.05$, n = 75) was observed between surface HPLC-derived TChl a and Fm-derived TChl a. It shows that Fm-derived TChl a is applicable in this study region. Subsurface TChl a maxima (SCM) were observed between 30–80 m, mostly below the mixed layer and above the euphotic depth" to the methods section.
**As suggested, the sentence "A linear regression of r2 = 0.89 ($p < 0.05$, n = 75) was observed between surface HPLC-derived TChl a and Fm-derived TChl a. It shows that Fm-derived TChl a is applicable in this study region." has been moved to Section 2.6, whereas the sentence "Subsurface TChl a maxima (SCM) were observed between 30–80 m, mostly below the mixed layer and above the euphotic depth" remained in this section as we believe**

**it is more appropriate to describe the HPLC-TChla in the pigment section.**

P12128 L27: change "shows" to "suggests", because there is no direct evidence of different strains.
**We have modified this sentence.**

P12129 L 9: Please define VAZ on first use.
**We have modified this sentence.**

P12129 L5-30: This section could do with a bit of work. To what extent did the changes in pigment concentrations reflect different community structure versus photo acclima- tion? Better use of the literature (e.g. Griffith and Vennell 2010 Nature Proceedings; Alderkamp 2011 DSRI) would help make the evidence for xanthophyll cycling much more convincing.
**We have modified Section 3.4.2 (now Section 3.4) by discussing response of phytoplankton to light looking at the different information from pigment, absorption and fluorescence data. As suggested, we have included results of PC (photosynthetic carotenoids) versus TChla and NPC (photoprotective carotenoids) versus TChla in correspondence to the fluorescence and phytoplankton absorption data and have added additional information on taxonomy vs photoacclimation in Section 3.4. We finalised the section by discussing the time scales of the response and driving main conclusions on photoadaptation versus photoacclimation of the phytoplankton in our studied regions (shallow versus deep ocean stations, surface layer versus SCM layer).**

P12130 L14-15: "For deeper offshore stations, aph were usually lower at the surface and increase with depths (Fig. 8c–e). This occurs when cells are acclimated to low irradiances at deeper depth, leading to subsequent increase in pigmentation and less efficient absorption per mass of pigment Falkowski et al. (1985)" The second sentence contradicts the first here. The data show an increase in a*ph with depth while Falkowski refers to a decrease in a*ph with depth as a result of the package effect. I am intrigued by the observed increase in a*ph with depth (in Fig 8), which is unusual and does not seem to fit with the other data presented (in stratified water columns with subsurface chlorophyll maxima containing larger cells, a*ph would be expected do decrease with depth). Is the data correct? If so, what is the explanation?
**We are grateful for this comment. We have carefully checked the aph and aph* values and found that aph data from those deeper depths were really low and noisy (at the detection limited). When then normalizing those data with the very low chl-a conc. measured at those specific depths the resulting aph* values became very high and probably not reliable. Therefore, we have removed those data from Fig 8., but have also added a few more figures to support our discussion in Section 3.4.**

Section 3.7: Better use of literature on mixing vs. acclimation timescales (of which there is a lot, e.g. by Cullen, Marra, Falkowski, Geider, Moore and many others) would help make this a more robust discussion.

**We have incorporated the discussion of mixing vs. acclimation time scales into Section 3.4.**

P12133 L26: What do you mean by “disappearance ratio”?
**We have changed it to “nutrient ratio”**

Figures: - Is a map needed in Fig 2 and 4? Can the information be combined into Fig 1? - Is it necessary to quote both the CTD number and the station number? I suggest using one or the other (or, better still, something more meaningful like latitude). Fig 7: please include units, g:g? mol:mol?
**We have modified Fig 2 and 4 according to the suggestion of Referee#1. As suggested, we have removed station number from all vertical profile figures. We have included units in Fig 7.**