

Interactive comment on “Seasonal occurrence of anoxygenic photosynthesis in Tillari and Selaulim reservoirs, Western India” by S. Kurian et al.

By Dr. Borrego

Referee’s comment:

The work by Kurian and co-workers describes the presence and dynamics of GSB populations in two Indian reservoirs through the analysis of several pigment biomarkers for these microorganisms (i.e. BChl e farnesyl-esterified homologs and isorenieratene). The work is interesting since it describes by first time the occurrence of these primary producers in these systems, providing estimations on their potential contribution to carbon fixation. In its present form, however, the manuscript contains several flaws and errors that must be corrected prior to its final acceptance. Below I have included a list of comments and suggestions for improvement, which should be carefully considered by the authors.

Reply:

We thank Dr. Borrego for his thorough review which has helped in improving the manuscript to a considerable extent. Most of his suggestions have been incorporated in the revision. The detailed response to his comments is as follows.

Referee Comment:

GENERAL COMMENTS

My first impression is that the authors have overstated the importance of their results. They should always remember that they have only analysed two reservoirs (and one of them was sampled only once!). These results may not be indicative for the rest of Indian reservoirs (whatever their number will be) and generalizations are risky, if not erroneous. The final sentence in the discussion section (although there are other examples throughout the MS) is clearly indicative of this: "...our studies reveal the importance of anoxygenic photosynthesis in Indian reservoirs during summer stratification." (Page 12168, Lines 10-11). Please, correct and be more cautious.

Reply:

Although we believe that similar conditions may also prevail in other reservoirs, following the referee’s advice we have toned down the implications of our results and made appropriate changes in the revision.

Referee’s Comment:

The discussion section must be considerably reduced. In my opinion it contains several paragraphs that are not a proper discussion of results but only general information on GSB ecology and physiology. Most of these sentences must be placed in the introduction or completely removed. Other paragraphs are clearly speculative and should be avoided.

Reply:

The Discussion section has been modified considering referee's comments and shortened to some extent.

Referee Comment: In my opinion, authors have misidentified the three BChl e homologues found in their samples. BChl e1 ([E,M]BChl eF) is usually absent in natural populations and it is only clearly traceable in laboratory cultures grown under high light intensities (Borrego and Garcia-Gil 1994; 1995). According to my experience, the proper identification would be BChl e2, BChl e3 and BChl e4. This identification agrees with the typical homolog composition of brown-coloured GSB thriving under low light conditions in natural habitats (Borrego et al., 1997) and, especially, to the m/Z molecular masses obtained by the authors (caption to Figure 4) and listed in previous works (Airs et al., 2001, Photosynth. Res. 70: 221-230; Glaeser et al., 2002, Arch. Microbiol. 177: 475-485). A simple comparison of these values yields the correct identification: BChl e2 (wrongly identified as e1 in the text) = [E,E]BChl eF (m/z = 821.5); BChl e3 (wrongly identified as e2 in the text) = [P,E]BChl eF (m/z = 835.5); and BChl e4 (wrongly identified as e3 in the text) = [E,E]BChl eF (m/z = 849.5). The correct m/z for BChl e1 = [E,M]BChl eF is 807-809 (Airs et al., 2001; Glaeser et al., 2002) is clearly below the masses of the homologues identified by the authors. Please correct throughout the manuscript and figures

Reply:

We thank the referee for proper identification of BChl e homologues. In the revised manuscript, BChl e isomers are properly identified (e2, e3 and e4) and also corrected in the table and figures.

Referee Comment:

- Some references cited in the text are not in the final list (see specific comments below).
- I suggest redrawing some of the Figures for better visualization/readability (see specific comments below).
- Although the MS is well written and understandable, authors should carefully check English language to avoid colloquial expressions and confusing sentence structures.

Reply: Accepted.

SPECIFIC COMMENTS

Abstract

Page 12154, Line 15: Replace "computed" by "estimated". Please provide conversion factors in M&M section.

Reply: Accepted.

Referee Comment:

P12154, L17-18: The sentence "These results highlight the importance..." clearly overstate the results (see previous comment). Please correct and be modest.

P12154, L23: Replace "noted" by "detected"

Reply: Accepted.

Referee Comment:

Introduction

P12155, L16-17: None of these works used LC-MS. Please, correct.

Reply: References have been changed in the revised manuscript.

P12155, L20: Replace "brown coloured ones" by "brown-coloured species"

Reply: Modified

P12156, L8: Reference by Narvenkar et al. 2011 is not in the final list.

P12155, L18: Reference by Shenoy et al., 2011 is not in the final list.

Reply: Since these manuscripts are still in preparation, the citation has been changed to 'unpublished data'.

Referee Comment:

Material and Methods

P12158, L5: The wavelengths used for detection (450 and 665 nm) correspond to the Soret and Qy bands of bacteriochlorophylls not to the excitation and emission since the authors used a PDA detector not a fluorescence one. Am I right?

Reply: We agree. Appropriate changes have been made in the revised manuscript.

Referees' comment:

P12159, L16: Please provide a valid reference for the Pfennig medium not the web address of the DSMZ!

Reply: Accepted.

P12159, L18: Replace "served" by "added"/"fed"

Reply: Accepted

P12159, L18: "... and other supplements..." is vague. Please, specify which were these "other supplements" and their final concentrations in the culture medium.

Reply: Appropriate changes made in the revised manuscript

P12159, L20: "...confirming the enrichment of the culture in GSB species".

Reply: Modified

Results

P12160, L5: "shoaled"? I don't understand the meaning here? Do you mean that the oxic/anoxic layer moved upward? Please, specify and correct if appropriate.

Reply: 'Shoaled' means 'to become shallow(er)'. Appropriate change has been made in the manuscript.

P12160, L21: The misidentification of BChl e homologs is a major issue to be corrected (see general comments above).

Reply: Corrected throughout the manuscript.

Discussion

Referee Comment:

P12162, L15: The whole discussion section must be shortened (see general comments above).

The Discussion section has been modified considering referee's comments and shortened to some extent.

Referee Comment:

P12165, Lines 14-29: The entire paragraph is clearly not a discussion of your results but some sort of review of results from other authors. Please, rewrite using only the information useful to discuss your results in a theoretical context. Also, "isorenieratene" is misspelled in this paragraph and below (isoreneiratene). Please, correct.

Reply: Accepted. Modifications have been made in the revised manuscript.

Referee's Comment:

P12167, L3: I think that it is probably better to integrate the BChl e concentration only in the anoxic compartment not from the surface to the bottom. I suggest recalculating these integrated values.

Reply: Standing stock of BChl e isomers has been recalculated only for the anoxic compartment and the text has been modified accordingly. However, the difference is small (2.27 g C m⁻² instead of 2.49 g C m⁻²).

Referee's Comment:

P12167, L10: "admittedly imprecise" is colloquial. Please, correct.

Reply: Modified in the revised manuscript.

P12167, L20-22: In this context "plant" Chl a is not appropriate, use "algal" instead.

Reply: Accepted

P12167, L23: ...chlorophyll concentrations not "levels".

Reply: Accepted

P12167, L25: Reference by Narvenkar et al. 2011 is not in the final list.

Reply: Changed to unpublished data

P12168, L24: "in a larger water compartment from 11 to 45 m depth..."

P12168, L5: "Previous studies showed that..."

P12168, L5: "microorganisms" delete the extra space.

Reply: Modified in the revised manuscript

P12168, L11: The final sentence is a clear overestimation of the results (see general comments).

Reply: Changed in the revised manuscript.

References

- Please review the reference list carefully. Reference must be cited in alphabetical order of the first author and then chronologically (correct the final set of references by Yacobi that are not chronologically sorted).

Reply: References are checked and modified as per referee's suggestion.

Figures

- Figures 2 and 3. Some issues must be addressed, namely:

- Figs. 2A/3A: Units for Dissolved Oxygen should be mg/l not ml/l. Am I right? If so it must be corrected in both the axis and the caption.

Reply: Dissolved oxygen concentration in water can be expressed in any of the following units: ml/l, mg/l, micromole/l and micromole/kg. We prefer ml/l, and so we have retained this unit. However, in the legend we have provided the conversion factor (1.43) from ml/l to mg/l.

Figs. 2B/3B. I suggest plotting the total concentration of BChl e (e2+e3+e4) since the individual homolog concentration does not provide any extra information using this type of plot. If the authors consider that the homolog composition of the population is an important issue to emphasize (as I believe), I suggest plotting the individual concentrations of homologs (or their relative abundance, in %, -probably better-) in a vertical bar plot comparing different dates for Tillari reservoir (2B and 3B) and Selaulim reservoir (6B).

Reply: We agree with the referee and in the revised manuscript, total BChl e concentration is shown in the figures 2B, 3B and 6B (vertical profiles) and the relative abundance of isomers is given in the text.

Figure 3C

- I suggest plotting light intensity in a logarithmic axis. In its present form the light extinction along depth is hardly visible, especially the change in light extinction (change in the slope of the curve) caused by the GSB population.

Reply: Light intensity has now been plotted on the logarithmic scale.

Figure 4: The identification of BChl e homologs is erroneous. Please, correct according to comments above.

Reply: Corrected throughout the manuscript.

- Figure 6: Same comments for 6A and 6B than those listed above for Figures 2 and 3.

Reply: Modified in the revised manuscript.