Biogeosciences Discuss., 8, 12153–12178, 2011
www.biogeosciences-discuss.net/8/12153/2011/
doi:10.5194/bgd-8-12153-2011
© Author(s) 2011. CC Attribution 3.0 License.

Biogeosciences Discussions

This discussion paper is/has been under review for the journal Biogeosciences (BG).
Please refer to the corresponding final paper in BG if available.

Seasonal occurrence of anoxygenic photosynthesis in Tillari and Selaulim reservoirs, Western India

S. Kurian[1], R. Roy[1,2], D. J. Repeta[2], M. Gauns[1], D. M. Shenoy[1], T. Suresh[1], A. Sarkar[1], G. Narvenkar[1], C. G. Johnson[2], and S. W. A. Naqvi[1]

[1]National Institute of Oceanography, CSIR, Goa 403 004, India
[2]Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

Received: 30 November 2011 – Accepted: 6 December 2011 – Published: 16 December 2011

Correspondence to: S. Kurian (siby@nio.org)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Phytoplankton and bacterial pigment compositions were determined by high performance liquid chromatography (HPLC) and liquid chromatography- mass spectrometry (LCMS) in two freshwater reservoirs (Tillari Dam and Selaulim Dam), which are located at the foothills of the Western Ghats in India. These reservoirs experience anoxia in the hypolimnion during summer. Water samples were collected from both reservoirs during anoxic periods while one of them (Tillari Reservoir) was also sampled in winter, when convective mixing results in well-oxygenated conditions throughout the water column. During the periods of anoxia (summer), bacteriochlorophyll (BChl) *e* isomers and isoreneiratene, characteristic of brown sulfur bacteria, were dominant in the anoxic (sulfidic) layer of the Tillari Reservoir under low light intensities. The winter observations showed the dominance of small cells of Chlorophyll-*b* containing green algae and cyanobacteria, with minor presence of fucoxanthin-containing diatoms and peridinin-containing dinoflagellates. Using total BChl-*e* concentration observed in June, the standing stock of brown sulfur bacteria carbon in the Tillari Reservoir was computed to be 2.4 $gC\,m^{-2}$, which is much higher than the similar estimate for carbon derived from oxygenic photosynthesis (0.82 $gC\,m^{-2}$). These results highlight the importance of anoxygenic photosynthetic biomass in tropical freshwater systems. The Selaulim Reservoir also displayed similar characteristics with the presence of BChl-*e* isomers and isorenieratene in the anoxic hypolimnion during summer. Although sulfidic conditions prevailed in the water column below the thermocline, the occurrence of photoautotrophic bacteria was restricted only to mid-depths (maximal concentration of BChl-*e* isomers was noted at 0.2 % of the surface incident light). This shows that the vertical distribution of photoautotrophic sulfur bacteria is primarily controlled by light penetration in the water column where the presence of H_2S provides a suitable biogeochemical environment for them to flourish.

1 Introduction

In stratified lakes having anaerobic hypolimnia, phototrophic anoxic bacteria have been known to contribute substantially to total primary production and biomass (Biebl and Pfennig, 1979; Hurley and Watras, 1991). These bacteria use sulfide and other reduced sulfur compounds and sometimes also iron sulfide as electron-donors and light as energy source for anoxygenic photosynthesis (Garcia-Gil et al., 1990; Imhoff, 1992). These bacteria are divided into the purple sulfur bacteria (PSB) and the green sulfur bacteria (GSB). Purple sulfur bacteria are generally pigmented with bacteriochlorophyll (BChl) *a* or *b* while green sulfur bacteria contain BChl-*c*, *d* or *e* along with their characteristic carotenoids. Green sulfur bacteria such as *Chlorobium (Cb.) phaeobacteroides* contain a large amount of aggregated BChl-*e* along with high content of carotenoids in the chlorosome and are brown colored due to the presence of BChl-*e* aggregates. While few other GSB such as *Cb. tepidum, Cb. limicola etc.* look green due to the presence of large aggregated BChl-*c* and *d*, with a low content of carotenoids (Hirabayashi et al., 2004). Recently, the application of HPLC and LC-MS has made the analysis of bacteriochlorophyll isomers more accurate and specific (Korthals and Steenbergen, 1985; Yacobi et al., 1990; Borrego et al., 1997). Importantly, accumulation of phototrophic bacteria close to the oxic-anoxic boundary layer depends strongly on the available light (Pfenning, 1989), which can even act as a limiting factor. In comparison with the green sulfur bacteria, the brown colored ones have been shown to live deeper in the water column where the available spectral range is limited to 400–600 nm (Vila and Abella, 1994).

There have been several studies of photoautotrophic sulfur bacteria in freshwater lakes (Takahashi and Ichimura, 1968; Yacobi et al., 1990; Garcia-Gil and Abella, 1992; Yacobi et al., 1996; Borrego et al., 1997; Vila et al., 1998; Yacobi and Ostrovski, 2008, 2011). Based on BChl-*e* pigments, Yacobi et al. (1990) reported the dominance of brown sulfur photosynthetic bacteria in the anoxic hypolimnion of Lake Kinneret, Israel. The isomers BChl-*e*1, *e*2, *e*3 and *e*4 were studied by Borrego et al. (1997) in several

meromictic and holomictic lakes of Europe and USA. Meromictic lakes have been found to support dense populations of anaerobic photosynthetic bacteria as the stagnant bottom waters are rich in nitrogen, phosphorus and sulfide with the species variability dependent on light availability and physicochemical characteristics of the lake (Garcia-Gil and Abella, 1992; Borrego et al., 1997).

India has a large number of natural freshwater lakes and man-made reservoirs created by damming rivers, some of which have recently been found to experience anaerobic conditions including sulphate reduction in the hypolimnia during summer (Narvenkar et al., 2011). We selected two such systems – the reservoirs of Tillari and Selaulim dams – to undertake a study of phytoplankton and bacterial pigments in Indian freshwater lakes, the results of which are being reported here. These reservoirs are located at the foothills of the Western Ghats in the states of Maharashtra (Tillari) and Goa (Selaulim) (Fig. 1). The former is currently being sampled by us at least once a month since March 2010. It was found to develop anoxic conditions in the hypolimnion during summer (May to July, 2010) that was terminated by the re-oxygenation of the hypolimnion in July/August (Shenoy et al., 2011). Convective mixing led to the prevalence of well-oxygenated conditions during the following winter. Based on HPLC and LC-MS analyses, we investigate the variability of phytoplankton and bacterial pigments during summer (May–July, 2010) in relation to the development of water column anoxia. We compare these data with one observation during winter (January, 2011). In Selaulim Reservoir, which has a smaller storage capacity (0.23×10^9 m^3 as compared to 0.45×10^9 m^3 for the Tillari Reservoir), observations were made only once – in May, 2010 – when intense anoxia prevailed in the hypolimnion. Finally, an attempt is made to quantify the relative contribution of oxygenic and anoxygenic photoautotrophs to microbial biomass during the summertime in the Tillari Reservoir.

2 Methodology

Water sampling in the reservoirs was carried out using 5 l Niskin samplers mounted on a nylon wire and fitted with reversing thermometers to measure temperature at different sampling depths. Subsamples for dissolved oxygen (DO) and hydrogen sulfide (H_2S) were collected carefully avoiding air-exchange and taken to the laboratory for analysis. Dissolved oxygen and H_2S were estimated following standard titrimetric Winkler and colorometric methods respectively (Grasshoff et al., 1983).

Optical parameters were derived using the radiative transfer simulation code Hydrolight Version 5.0 (http://www.sequoia.com). The inputs to the simulations were absorption and beam attenuation at nine wavelengths measured in situ using the AC-9 spectrophotometer (WETLabs, Inc.) and solar irradiance at the surface. The downwelling solar irradiance for the day was derived using the solar irradiance model (Gregg and Carder, 1990). Aerosol optical depth at 550 nm, Angstrom exponent, ozone and pressure for the day were derived from ocean color satellite MODIS Aqua available from Giovanni GES-DISC (Goddard Earth Sciences Data and Information Services Center) (http://disc.sci.gsfc.nasa.gov/giovanni). Other meteorological parameters such as the wind speed and humidity were derived using climatological data over the region. The volume scattering function for the Hydrolight simulations was from Petzold (1972).

For the pigment analysis, water samples were collected in amber colored bottles and were filtered (~0.5 to 1 l) on Whatman glass fibre filters (GF/F; pore size 0.7 µm) under dark conditions and stored at −80 °C until the analysis. The frozen filters were extracted within 2–3 weeks of collection at 0 °C for 2 min in 3 ml of 100 % methanol using ultrasonic dismembrator model 100 (Fisher Scientific) at 23 kHz. The extracts were filtered using a Teflon syringe cartridge (Sartorius Minisart, pore size 0.45 mm, diameter 25 mm) to remove any cellular debris, and analyzed using HPLC (Agilent Technologies) at the National Institute of Oceanography (NIO), Goa using an Eclipse XDB C8 column. Pigments were separated following a slight modification of the procedure as detailed in Roy et al. (2006). Briefly, elution was performed at a rate of 1.1 ml min^{-1} using a linear

gradient program over 45 min as follows: initially 5 % of solvent B (methanol) followed by a linear gradient over 22 min to 95 % B, with an isocratic hold for 13 min at 95 % B until 35 min, which returned to initial condition of 5 % B prior to the next analysis. Solvent A was 70/30: methanol /0.5 M ammonium acetate and the column was maintained at 60 °C during the analysis. The eluting pigments were detected at 450 and 665 nm (excitation and emission) by the diode array detector. Commercially available standards obtained from DHI Inc. (Denmark) were used for the identification and quantification of pigments including both chlorophylls and carotenoids. Identification was based on the retention time and visible spectra matching. Pigment concentrations were computed from the peak areas as detailed in Roy et al. (2006).

Bacteriochlorophylls were identified and quantified by HPLC and LC-MS at Woods Hole Oceanographic Institution (WHOI), USA. Extraction of samples was done using acetone in ultrasonic bath, dried and re-dissolved in methanol and analysed by HPLC (Agilent Technologies) equipped with C-18 column (15 cm × 4.6 mm, 3 µm, Supelco Inc). Elution was performed at a rate of 1.5 ml min^{-1} for 60 min using a linear gradient program with the eluent composition varying as follows: 100 % solvent A (80/20: MeOH/0.05 M ammonium acetate) to begin with, followed by a linear gradient over 20 min to 100 % B (80/20: MeOH/acetone); an isocratic hold for 25 min with 100 % B for elution of all the major pigments then returning to 100 % A for 5 min; finally, 10 min hold with 100 % A to equilibrate the column before next analysis. Pigments were detected at 410, 442, 470 and 660 nm by the diode array detector (DAD).

Samples were also analyzed by LC-MS (Agilent Technologies) using an atmospheric pressure chemical ionization (APCI) source operated in the positive ion mode, scanning from 300 to 1200 m/z. The same column and eluting solvents were used and the peaks were confirmed based on mass spectra in full scan and also in the selected ion monitoring (SIM) mode. The conditions used for LC-MS analysis: vaporizer temperature 400 °C, drying gas temperature 300 °C and discharge current 3 µA. Bacteriochlorophyll standards were extracted from particulate matter from Salt Pond, USA, in which BChl-*e* isomers were dominant. BChl-*e*1, *e*2 and *e*3 isomers were separated

using fraction collector, and quantified by spectrophotometic measurements assuming a molar extinction coefficient 48.9 mM cm^{-1} (Borrego et al., 1999a). Multi-point calibration table was prepared for BChl-*e*3 isomer and same calibration was used for BChl-*e*1 and *e*2 isomers as they are different only by CH_2 groups. Isorenieratene was quantified based on β–carotene calibration and was present in the anoxic samples.

Sub-samples (1.5 ml) were also collected and preserved with glutaraldehyde (0.25 % final concentration), frozen in liquid nitrogen and then stored at −80 °C for flow cytometric analysis at NIO. These samples were thawed at room temperature and analysed using a FACS Calibur Flow Cytometer following Marie et al. (1997) to obtain absolute counts. A combination of the FSC (forward scatter)-SSC (side scatter), the SSC-FL3 (red fluorescence) and the FL2 (orange fluorescence) – FL3 plots were used to differentiate populations in the sample (Casamayor et al., 2007). The data was analyzed using CYTOPC software (Vaulot, 1989). A culture of photosynthetic sulfur bacteria isolated from the seasonal anoxic waters off Goa (September, 2006) at a depth of 25 m was run to identify this form from other fields. The culture was maintained in Pfenning's medium II (http://www.dmsz.de) at a pH of 6.6–6.8 which was previously purged with nitrogen and later saturated with CO_2 gas. The non-axenic culture medium was periodically served with sodium sulfide and other supplements to replenish the photosynthetic electron donors. HPLC analysis of the culture (brown in colour) showed the dominance of different BChl-*e* isomers, confirming the presence of GSBs.

3 Results

3.1 Tillari Reservoir

3.1.1 Hydrographic conditions

During May and June, the water column was strongly stratified, with a surface-to-bottom temperature difference of ∼7 °C (Figs. 2a and 3a), and contained distinct

biogeochemical regimes i.e., oxic epilimnion separated from the anoxic hypolimnion. In May, DO was close to detection limit at and below 20 m; H_2S was present at and below 25 m, with concentrations reaching up to 8.3 µM close to the bottom (∼50 m, Fig. 2a). Similar conditions prevailed in June as well with the anoxic layer appearing to have shoaled slightly when compared to the previous month. The highest H_2S concentration observed on this occasion was 9 µM (Fig. 3a). During July, H_2S concentration in the anoxic bottom waters had declined to 3.2 µM (data not shown). Stratification broke down during winter (January, 2011), with the surface to bottom temperature difference being only ∼1 °C, and the water column was well oxygenated. In June, computed light irradiance at the surface was 1523.4 µmol photon m^{-2} s^{-1}, which decreased to 0.0565 µmol photon m^{-2} s^{-1} at the bottom of the reservoir.

3.1.2 Phytoplankton pigments

In the Tillari Reservoir, chlorophyll-*a* (Chl-*a*) concentration was 2.06 µg l^{-1} at the surface in May, decreasing to 0.04 µg l^{-1} in bottom waters (50 m; Table 1). Surface Chl-*a* decreased to 1.76 µg l^{-1} in June and to 0.55 µg l^{-1} in July (Table 1). During most observations, Chl-*a* concentrations remained low in the metalimnion; however, when anoxic conditions prevailed this layer became enriched with BChl-*e* isomers (Table 1) and most other pigments were either absent or below detection limit. During summer stratification (May–July, 2010), BChl-*e*1,*e*2 and *e*3 isomers were identified based on their UV/Vis spectra, molecular ions, and major fragment ions from APCI-LC-MS (Fig. 4a, b, c, d). In the anoxic waters, bacteriochlorophyll-*e*1,*e*2 and *e*3 isomers were dominating and represent the major peaks in Fig. 4a. The m/z molecular ions of these isomers (821.5, 835.5 and 849.5) and respective fragment ions (by the loss of H_2O molecule; −18AMU) were identified in each spectrum (Fig. 4b, c and d; Chen et al., 2001). These isomers were present close to the oxic-anoxic interface of this reservoir during summer stratification, with the highest concentration (∼3 µg L^{-1}) occurring in June (Fig. 3b). BChl-*e*1 varied from below detection limit (BDL) to 2 µg l^{-1} with a mean concentration of 0.06 µg l^{-1}. Similarly, BChl-*e*2 and *e*3 values ranged between

BDL and 3.97 µg l^{-1}, and BDL and 3.52 µg l^{-1}, with means of 0.12 and 0.09 µg l^{-1} respectively (Table 1). Isorenieratene, a carotenoid characteristic of brown sulfur bacteria was also identified based on mass spectrum (m/z 529.3) at the depths where BChl-*e* isomers were present in June (range BDL –0.19 µg L^{-1}, Fig. 3b).

The highest Chl-*a* (2.26 µg l^{-1}) concentration was observed in January 2011 (Table 1). Apart from Chl-*a*, other chemotaxonomic pigments identified were chlorophyll-*b*, zeaxanthin, fucoxanthin which represent green algae, cyanobacteria and diatoms respectively. Other minor pigments associated with the green algae (Chlorophyceace) such as neoxanthin, violaxanthin and lutein were also found during the January observation (Table 1). Chlorophyll-*b* showed a temporal variation similar to Chl-*a*, but the concentrations were lower by an order of magnitude. The highest concentration (0.28 µg l^{-1}) was observed in winter. Zeaxanthin, a marker pigment of cyanobacteria was present in higher concentrations in May (surface) and January and contributed significantly to the biomass structure in the Tillari Reservoir (Table 1). Fucoxanthin also showed similar distribution pattern, but its concentration was lower than those of zeaxanthin and Chl-*b*. Peridinin, a marker pigment of dinoflagellates was present during May–August. Nonmarker photoprotective xanthophylls such as diadinoxanthin and diatoxanthin were also present in these samples; with comparatively higher concentrations in January (0.014 and 0.012 µg l^{-1}, respectively) (Table 1).

3.1.3 Flow cytometric identification and enumeration of photosynthetic sulfur bacteria

Flow cytometric analyses of samples collected from Tillari Reservoir in June was carried out for identifying the dominant groups in the anoxic waters. Based on photosynthetic sulfur bacteria culture (Fig. 5a), the suboxic-anoxic zone between 11 and 35 m was found to be dominated by cells of photosynthetic sulfur bacteria (23 m is shown in Fig. 5c). This group showed clear sub-surface maxima with peak abundance (1.7×10^8 l^{-1}) between 15 and 23 m and negligible concentrations at the surface and

near bottom waters (Fig. 5b and d); a trend similar to HPLC based bacteriocholorophyll-*e* distribution.

3.2 Selaulim Reservoir

The Selaulilm Reservoir is shallower (maximum depth ~20 m at the time of our sampling) as compared to the Tillari Reservoir. On the only occasion it was sampled (in May 2010), the water column was strongly stratified with the surface-to-bottom temperature difference of ~6 °C (Fig. 6a). The DO disappeared at 9 m, and H_2S was present below 12 m with the highest concentration (5.7 µM) occurring at 18 m (Fig. 6a). Chlorophyll-*a* and zeaxanthin were restricted to the epilimnion, although their concentrations were much lower than the Tillari Reservoir. BChl-*e*1,*e*2 and *e*3 isomers were present at 9 m and below all the way down to the deepest sampling depth (Fig. 6b). The highest concentrations of total BChl e isomers (6.4 µg l^{-1}) were found at 15 m where H_2S concentration was 1.83 µM. Isoreneiratene also showed higher concentration (0.39 µg l^{-1}) at this depth which implies the dominance of photosynthetic sulfur bacteria (Fig. 6b).

4 Discussion

4.1 Comparison of phytoplankton assemblages during summer stratification and winter mixing in the Tillari Reservoir

Many tropical reservoirs are known to experience anoxic conditions in the hypolimnia during summer stratification that is conducive for, among other phenomena, anoxygenic photosynthesis. However, no such observations are presently available from Indian fresh water systems.

The water column in the Tillari Reservoir is strongly stratified from March to June, whereas lower atmospheric temperatures and wind convective mixing ensures vertical homogenization during winter (December–February). This not only results in the

establishment of contrasting redox regimes (anoxia in the hypolimnion during summer), but also differential availability of nutrients in the surface layer (macro nutrient limitation during summer). During peak stratification, the epilimnion of the Tillari Reservoir is <10 m thick, such that the metalimnion and part of the hypolimnion receive significant amount of sunlight (Fig. 3c). These changes in physico-chemical characteristics of the reservoir are systematically reflected in the phytoplankton community structure. During the period of strong stratification (May–July), the community within the epilimnion mainly consists of smaller nanoflagellates, cyanobacteria, diatoms and dinoflagellates as indicated by the presence of zeaxanthin, Chl-*b*, fucoxanthin, peridinin and alloxanthin (Table 1). There is also evidence for anoxygenic photosynthesis (based on presence of BChl-*e*) in the metalimnion and hypolimnion as discussed in Sect. 4.2. The HPLC based pigment data is consistent with the flow cytometry data. Earlier work of Casamayor et al. (2007) shows the potential of flow cytometry as a fast tool for population identification and enumeration of photosynthetic sulfur bacteria. Using this tool the natural samples from the Tillari Reservoir were analysed against the mixed culture of photo-autotrophic sulfur bacteria, which showed that these bacteria are the dominant constituent of the autotrophic picoplankton community at sub-surface (ca. 15–27 m) depth (suboxic/anoxic waters of the metalimnion and upper hypolimnion; Fig. 5). Maximum abundances of these bacteria ($1.7 \times 10^8\,l^{-1}$ at 23 m) found in the present study were much lower than those of brown species of green sulfur bacteria ($8.6 \times 10^8\,l^{-1}$) reported from the Lake Vilar at shallow depth (5 m below surface) under sulfidic condition (Bañeras and Garcia-Gil, 1996).

In January, phytoplankton community structure in the Tillari Reservoir was dominated by Chl-*b*-containing green algae and cyanobacteria with minor presence of diatoms in the epilimnion; however, these pigments were also present in the deeper waters (Table 1) presumably due to vertical mixing. Although zeaxanthin is present in green algae as well as cyanobacteria, the presence of other accessory marker pigments such as violaxanthin, lutein and neoxanthin, not found in cyanobacteria confirm the presence of green algae (Jeffrey and Vesk, 1997). Phaeophytin-*a* concentration in bottom waters

was high, between May and July (0.11 to 0.45 $\mu g\,l^{-1}$), compared to that observed during January (BDL-0.42 $\mu g\,l^{-1}$). This could be attributed to better preservation in anoxic waters. Such observations have previously been reported from the Black Sea and other anoxic zones (Anderson et al., 1994; Chen et al., 2001; Squier et al., 2002). A recent study of pigment composition in Petit Saut, a tropical reservoir in French Guiana, showed the dominance of Chlorophyceae (Chl-*a*, Chl-*b* and lutein) in the oxic epilimnion, and anoxygenic photo-autotrophic bacteria below the oxycline as revealed by BChl-*c* and BChl-*d* (Junet et al., 2009). An example of how the community structure of phytoplankton is dependent on nutrient availability is provided by the results of Zhu et al. (2010) from Taihu Lake (China) where Chlorophyta were found to be predominant at high concentrations of nitrogen and phosphorus, whereas cyanobacteria dominated in waters having low concentrations of these nutrients. Our data suggest co-existence of both these groups in the Tillari Reservoir.

4.2 Anoxygenic photosynthesis in Tillari and Selaulim reservoirs during summer stratification

In the Tillari Reservoir, the highest concentration of BChl-*e* ($e1 + e2 + e3 = 9.5\,\mu g\,l^{-1}$) was found in June at 23 m where the H_2S concentration was 0.42 µM (Fig. 3a and b). These pigments were, however, detectable even at 11 m depth, where the water was suboxic ($0.17\,ml\,O_2\,l^{-1}$). The H_2S concentration increased to 9 µM below 23 m, but the concentration of BChl-*e* was lower (Fig. 3b), ostensibly due to the reduced light field. The computed light irradiance data show that around 1 % of the surface incident light reached 11 m where BChl-*e* isomers first appeared, and only 0.2 % of the surface incident light reached 23 m where the maxima in BChl-*e* isomers were located (Fig. 3c). Garcia-Gil and Abella (1992) studied photo-autotrophic bacterial populations in Spanish lakes and found the amount of light reaching the oxic-anoxic boundary to be the most important factor controlling their populations. However, they reported higher light intensities at the oxyclines of these lakes where BChl-*a* and *e* isomers were found

in high concentrations. In another study (one of the lakes in Lake Banyoles), Borrego et al. (1999b) observed high concentration of BChl-*e* isomers (up to 200 µg l^{-1}) at ~18 m depth and as summer stratification proceeded, the population of *C. pheobacteroides* (bacterial plate) moved to shallower depth (~17 m). They have explained the upward migration from 18 m to 16.5 m to increase the intensity of light at the bacterial plate corresponding to a change from 0.004 to 0.05 % of the surface incident light. These results illustrate the adaptability of the anoxygenic photosynthetic community to very low incident light levels similar to those encountered in the present study.

Although there are no optical data from the Selaulim Reservoir, the vertical distribution patterns of H_2S and BChl-*e* isomers, described in Sect. 3.2, are very similar to those observed in the Tillari Reservoir and it is reasonable to conclude that the anoxygenic bacterial population here is also controlled by the combined availability of free sulfide and sunlight.

Vila and Abella (1994) compared the photo-autotrophic bacterial populations of various lakes and showed that the brown- and green-pigmented groups of Chlorobiaceae have depth-dependent differential distribution patterns. Based on statistical analyses they demonstrated that the green-colored species, dominate at shallow oxic/anoxic boundaries, and are correlated to spectral intensity of long wavelength radiation, while the brown-colored species are correlated to spectral intensity in the central part of the spectrum (400–600 nm) with the maximum irradiance at 570–580 nm due to the presence of carotenoid isoreneiratene (Borrego et al., 1997). The BChl-*e* homologue distribution does not depend directly on light absorption, as in most of the studied lakes photons reaching the bacterial layer fall between 500–600 nm, which is not available for BChl harvesting but suitable for isoreneiratene (Vila and Abella, 1994), a carotenoid characteristic of brown colored sulfur bacteria. We also measured isoreneiratene in Tillari Reservoir during our June observation (Fig. 3b), which showed similar vertical profile of BChl-*e* isomers. Isoreneiratene was found to be present in Selaulim Reservoir also with highest concentration of 0.39 µg l^{-1} at 15 m (Fig. 6b) where BChl-*e* isomers showed the highest concentration. This further supports the presence of anoxygenic

photosynthesis in these reservoirs during summer stratification.

Highly alkylated bacteriochlorophyll-*e* isomers (*e*2, *e*3 and *e*4) have been found to be abundant in meromictic and holomictic lakes in Spain and USA (Borrego et al., 1997), where sulphide concentrations were very high (upto 2000 µM). It has been reported that a high concentration of alkylated, long-wavelength absorbing pigments improve energy transfer and increase absorption cross-section of photosynthetic systems (Fetisova et al., 1988; Borrego et al., 1997). In both Tillari and Selaulim reservoirs, we found bacteriochlorophyll-*e*1, *e*2 and *e*3 isomers, but the *e*4 isomer was absent (Fig. 4). The maximum sulphide concentration observed in these systems was ~9 µM and 5.7 µM, respectively (Figs. 2a and 6a). Hence, the dominant species of photosynthetic bacteria depends mainly on physico-chemical characteristics of the system (sufficient light and optimum sulphide concentration). Our results are similar to those of Borrego et al. (1997) who observed that the highly alkylated isomer (BChl-*e*4) was dominant in meromictic water bodies where sharp chemical gradient and strong reducing conditions prevailed (with sulphide occurring in millimolar level), while BChl-*e*3 isomer was abundant in holomictic bodies with broad physical and chemical gradients and low sulphide concentrations.

4.3 Standing stocks of oxygenically- and anoxygenically-photosynthesized carbon in the Tillari Reservoir

Earlier estimates of carbon fixation in lakes by photosynthetic sulfur bacteria vary widely depending on sulphide and light availability (Parkin and Brock, 1980 and references therein). In the present study, the total concentration of bacteriochlorophyll-*e* isomers was used as a proxy of biomass of green sulfur bacteria. Yacobi and Ostrovski (2011) reported photosynthesis by microalgae and cyanobacteria to be the major source of organic particles in the epilimnion of Lake Kinneret, while green sulfur bacterium (*Chlorobium phaeobacteroides*) was found in the anoxic layer throughout periods of stratification (Bergstein et al., 1979). In the Tillari Reservoir too bacteriochlorophyll-*e*1, *e*2 and *e*3 isomers were the dominant pigments in the anoxic

hypolimnion during summer stratification, while Chl-*a* was largely restricted to the epilimnion. We calculated the total BChl-*e* standing stock (mg m^{-2}) for the June observation by integrating the total BChl-*e* concentration from the surface to 45 m depth. The column-integrated BChl-*e* (124.6 mg m^{-2}) when combined with the published value of the BChl-*e*: C ratio (1:20 – Oelze and Golecki, 1988; Yacobi and Ostrovski, 2008, and references therein) suggests a standing stock of 2.4 g m^{-2} of anoxygenically photosynthesized carbon. For comparison, the standing stock (Chl-*a* 20.45 mg m^{-2}) of oxygenically photosynthesized carbon similarly calculated using a Chl-*a*: C ratio of 1:40 (Cole et al., 2002) for the June observation comes to 0.82 gC m^{-2}. These estimates, admittedly imprecise, nevertheless highlight the important – indeed dominant – contribution of anoxygenic photosynthetic biomass during the period of anoxia. In an earlier study by Takahashi and Ichimura (1968) made in stratified lakes in Japan, organic matter synthesized by photosynthetic sulfur bacteria in the reducing zone was reported to contribute 9–25 % of the total annual production in lakes rich in H_2S and 3–5 % in lakes poor in H_2S. In another study by Steenbergen (1982) in the lake Vechten (Netherlands), the photosynthetic sulfur bacteria accounted for 3.9 to 17.5 % of total daily productivity in the pelagial zone.

In Japanese lakes (Takahashi and Ichimura, 1968), mixed populations of green and purple sulfur bacteria produce bacterial chlorophyll concentrations as high as 1000 µg l^{-1} when these are abundant, whereas plant chlorophyll did not exceed 100 µg l^{-1} in the oxic zone. The column-integrated stocks of bacterial chlorophylls were 3–9 times those of plant chlorophyll, although the layer containing these bacteria was only 2 to 3 m thick (Takahashi and Ichimura, 1968). The chlorophyll levels in the Tillari Reservoir (both BChl-*e* and Chl-*a*) are not nearly this high, presumably due to moderate nutrient concentrations (Narvenkar et al., 2011). Another study in Lake Arcas (Spain), Camacho et al. (2000) reported high population densities of phototrophic micro organisms at the oxic-anoxic interface with high concentration of bacteriochlorophyll-*a* (up to 381 µg l^{-1}). We calculated the total BChl-*a* standing stock in Lake Arcas following similar calculation done for our study by integrating BChl-*a* concentration from

surface to 9.8 m depth, which accounted for 108.1 mg m^{-2}, while the standing stock of Chl-*a* seems to be much lower (72.1 mg m^{-2}; Camacho et al., 2000). This is consistent with our observation in Tillari Reservoir during summer. BChl-*e* isomers were present in wide depth zone ranging between 11 m and 45 m in Tillari Reservoir (June), though their concentrations were comparatively lower (max. 9.5 µg l^{-1} for $e1 + e2 + e3$). Several studies reported earlier show that these photo-autotrophic micro organisms are restricted to narrow depth ranges at the chemocline and their concentrations are highly variable depending on the physico- chemical characteristics of the lakes (Takahashi and Ichimura, 1968; Camacho and Vicente, 1998; Borrego et al., 1999b; Camacho et al., 2000; Rimmer et al., 2008). However, our studies reveal the importance of anoxygenic photosynthesis in Indian reservoirs during summer stratification.

Acknowledgements. The authors wish to thank the Director, NIO and managements of the Tillari and Selaulim reservoirs for permitting us to carry out the observations. We are grateful to H. Naik for logistical support and to H. Dalvi, A. Methar and B. R. Thorat for their kind assistance during sampling. S. Patil is acknowledged for his assistance in flow cytometric analysis. Financial support for this work was provided by the Council of Scientific & Industrial Research (CSIR) and Ministry of Earth Sciences (MoES). S. Kurian acknowledges POGO-SCOR for financial support to visit WHOI. R. Roy, G. Narvenkar and A. Sarkar received fellowship support from CSIR.

References

Anderson, R. F., Lyons, T. W., and Cowie, G. L.: Sedimentary record of a shoaling of the oxic/anoxic interface in the Black Sea, Mar. Geol., 116, 373–384, 1994.

Bañeras, L. and Garcia-Gil, L. J.: Role of photosynthetic microbial populations in the phosphorous exchange through the oxic-anoxic boundary in a meromictic eutrophic lake, Arch. Hydrobiol. Spec. Issues Adv. Limnol., 48, 171–181, 1996.

Bergstein, T., Henis, Y., and Cavari, B. Z.: Investigations on the photosynthetic sulfur bacterium Chlorobium phaeobacteroides causing seasonal blooms in Lake Kinneret, Can. J. Microbiol., 25, 999–1007, 1979.

Biebl, H. and Pfennig, N.: Anaerobic CO_2 uptake by phototrophic bacteria: A review, Arch. Hydrobiol. Beih. Ergeb, Limnol, 12, 48–58, 1979.
Borrego, C. M., Garcia-Gil, L. J., Vila, X. P., Figueras, J. B., and Abella, C. A.: Distribution of bacteriochlorophyll homologues in natural populations of brown-colored phototrophic sulfur bacteria, FEMS Microbiol. Ecol., 24, 301–309, 1997.
Borrego, C. M., Arellano, J. B., Abella, C. A., Gillbro, T., and Garcia-Gil, L. J.: The molar extinction coefficient of bacteriochlorophyll-*e* and the pigment stoichiometry in *Chlorobium phaeobacteroides*, Photosynth. Res., 60, 257–264, 1999a.
Borrego, C. M., Bañeras, L., and Garcia-Gil, L. J.: Temporal variability of Chlorobium phaeobacteroides antenna pigments in a meromictic karstic lake, Aquat. Microb. Ecol., 17, 121–129, 1999b.
Camacho, A. and Vicente, E.: Carbon photoassimilation by sharply stratified phototrophic communities at the chemocline of Lake Arcas (Spain), FEMS Microbiol. Ecol., 25, 11–22, 1998.
Camacho, A., Vicente, E., and Miracle, M. R.: Spatio-temporal distribution and growth dynamics of phototrophic sulfur bacteria populations in the sulfide-rich Lake Arcas, Aquat. Sci., 62, 334–349, 2000.
Casamayor, E. O., Ferrera, I., Cristina, X., Borrego, C. M., and Gasol, J. M.: Flow cytometric identification and enumeration of photosynthetic sulfur bacteria and potential for ecophysiological studies at the single-cell level, Environmental Microbiol., 9, 1969–1985, 2007.
Chen, C., Bianchi, T. S., McKee, B. A., and Bland, J. M.: Historical trends of hypoxia on the Louisiana shelf: Application of pigments as biomarkers, Org. Geochem., 32, 543–561, 2001.
Cole, J. J., Carpenter, S. R., Kitchel, J. F., and Pace, M. L.: Pathways of Organic Carbon Utilization in Small Lakes: Results from a Whole-Lake 13C Addition and Coupled Model, Limnol. Oceanogr., 47, 1664–1675, 2002.
Fetisova, Z. G., Freiberg, A. M., and Timpmann, K.-E.: Long-range molecular order as an efficient strategy for light harvesting in photosynthesis, Nature, 334, 633–634, 1988.
Garcia-Gil, L. J. and Abella, C. A.: Population dynamics of phototrophic bacteria in three basins of Lake Banyoles (Spain), Hydrobiologia, 243/244, 87–94, 1992.
Garcia-Gil, L. J., Sala-Genoher, L., Esteva, J. V., and Abella, C. A.: Distribution of iron in Lake Banyoles in relation to the ecology of purple and green sulfur bacteria, Hydrobiologia, 192, 259–270, 1990.
Grasshoff, K., Erhardt, M., and Kremiling, K.: Methods of seawater analysis, Verlag Chemie, 419 pp, 1983.

Gregg, W. W. and Carder, K. L.: A simple solar irradiance model for cloudless maritime atmospheres, Limnol. Oceanogr., 35, 1657–1675, 1990.
Hirabayashi, H., Ishii, T., Takaichi, S., Inoui, K., and Uejara, K.: The Role of carotenoids in the photoadaptation of the brown colored sulfur bacterium Chlorobium phaeobacteroides, Photochem. Photobiol., 79, 280–285, 2004.
Hurley, J. P. and Watras, C. J.: Identification of bacteriochlorophylls in lakes via reverse-phase HPLC, Limnol. Oceanogr., 36, 307–315, 1991.
Imhoff, J. F.: Taxonomy, phylogeny and general ecology of anoxygenic phototrophic bacteria, in: Biotechnology Handbook Photosynthetic Prokaryotes, edited by: Carr, N. G. and Mann, N. H., London & New York, Plenum, 53–92, 1992.
Jeffrey, S. W. and Vesk, M.: Introduction to marine phytoplankton and their pigment signatures, in: Phytoplankton Pigments in Oceanography, edited by: Jeffrey, S. W., Mantoura, F. C., Wright, S. W., UNESCO Publishing, Paris, 85–126, 1997.
Junet, A. de., Abril, G., Guérin, F., Billy, I., and Wit, R. de.: A multi-tracers analysis of sources and transfers of particulate organic matter in a tropical reservoir (Petit Saut, French Guiana), River Res. and Appli., 25, 253–271, 2009.
Korthals, H. J. and Steenbergen, C. L. M.: Separation and quantification of pigments from natural phototrophic microbial populations, FEMS Microbiol. Ecol., 31, 177–185, 1985.
Marie, D., Partensky, F., Jacquet, S., and Vaulot, D.: Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I, Appl. Environ. Microbiol., 63, 186–93, 1997.
Oelze, J. and Golecki, J. R.: Growth rate and the control of development of the photosynthetic apparatus in Chloroflexus aurantiacus as studied on the basis of cytoplasmatic membrane structure and chlorosome size, in: Green Photosynthetic Bacteria, edited by: Olson, J. M., Ormerod, J. G., Amesz, J., Stackebrandt, E., and Trüper, H. G, EMBO Workshop, Nyborg, Denmark, 35–42, 1988.
Parkin, T. B. and Brock, T. D.: Photosynthetic bacterial production in lakes: The effects of light intensity, Limnol. Oceanogr., 25, 711–718, 1980.
Petzold, T. J.: Volume scattering functions for selected ocean waters, Scripps Inst. Oceanogr., Report SI0, 72–78, 1972.
Pfennig, N.: Ecology of phototrophic purple and green sulfur bacteria, in: Auto-trophic bacteria, edited by: Schlegel, H. G. and Bowien, B., Springer Verlag, New York, 97–116, 1989.
Rimmer, A., Ostrovsky, I., and Yacobi, Y. Z.: Light availability for Chlorobium phaeobacteroides

development in Lake Kinneret, Jr. Plankton Res., 30, 765–776, 2008.
Roy, R., Prathihary, A., Mangesh, G., and Naqvi, S. W. A.: Spatial variation of phytoplankton pigments along the south west coast of India, Estuar. Coast. Shelf Sci., 69, 189–195, 2006.
Squier, A. H., Hodgson, D. A., and Keely, B.: Sedimentary pigments as markers for environmental change in an Antarctic lake, Org. Geochem., 33, 1655–1665, 2002.
Steenbergen, C. L. M.: Contribution of photosynthetic sulfur bacteria to primary production in Lake Vechten, Hydrobiologia, 95, 59–64, 1982.
Takahashi, M. and Ichimura, S.: Vertical distribution and organic matter production of photosynthetic sulfur bacteria in Japanese lakes, Limnol. Oceanogr., 13, 644–655, 1968.
Vaulot, D.: CYTOPC: Processing software for flow cytometric data, Signal and Noise, 2, 8 pp., 1989.
Vila, X. and Abella, C. A.: Effects of light quality on the physiology and the ecology of planktonic green sulfur bacteria in lakes, Photosynth. Res., 41, 53–65, 1994.
Vila, X., Abella, C. A., Figueras, J. B., and Hurley, J. P.: Vertical models of phototrophic bacterial distribution in the metalimnetic microbial communities of several freshwater North-American kettle lakes, FEMS Microbiol. Ecol., 25, 287–299, 1998.
Yacobi, Y. Z. and Ostrovsky, I.: Downward flux of organic matter and pigments in Lake Kinneret (Israel): relationships between phytoplankton and the material collected in sediment traps, Jr. of Plankton Res., 30, 1189–1202, 2008.
Yacobi, Y. Z. and Ostrovsky, I.: Sedimentation of photosynthetic pigments during the bloom of the green sulfur bacterium *Chlorobium phaeobacteroides* in Lake Kinneret: spatial patterns, Hydrobiologia, 660, 117–124, 2011.
Yacobi, Y. Z., Eckert, W., Trueper, H. G., and Berman, T.: High performance liquid chromatography detection of phototrophic bacterial pigments in aquatic environments, Microbial Ecology, 19, 127–136, 1990.
Yacobi, Y. Z., Pollingher, U., Gonen-Zurgil, Y., Gerhardt, V., and Sukenik, A.: HPLC analysis of phytoplankton pigments from Lake Kinneret with special reference to the bloom-forming dinoflagellate Peridinium gatunense (Dinophyceae) and chlorophyll degradation products, Jr. Plankton Res., 18, 1781–1796, 1996.
Zhu, W., Wan, L., and Zhao, L.: Effect of the nutrient level on phytoplankton community structure in different water bodies, Jr. Environmental. Sci., 22, 32–39, 2010.

Table 1. Concentration of pigments ($\mu g\,l^{-1}$) in the Tillari Reservoir during May, June, July, 2010 and January, 2011. Abbreviations are as follows: Total Chlorophyll (TChl), Bacteriochlorophyll (BChl), Phaeophytin (Phaeo), Isorenieratene (Isoren), Zeaxanthin (Zea), Peridinin (Per), Fucoxanthin (Fuco), Alloxanthin (Allo), Total carotene (Tcar), Neoxanthin (Neo), Violaxanthin (Viola), Lutein (Lut), Diadinoxanthin (Diad), Diatoxanthin (Diat). – refers "Below Detection Limit". Chlorophylls, BChls and isoren were measured at WHOI, while other carotenoids were quantified at NIO.

Depth (m)	TChl-*a*	Chl-*b*	BChl-*e*1	BChl-*e*2	BChl-*e*3	Phaeo-*a*	Isoren	Zea	Per	Fuco	Allo	Tcar	Neo	Viola	Lut	Diad	Diato
Field Trip 12/05/2010																	
0	2.06	0.13	–	–	–	0.02	–	0.17	0.03	0.01	0.003	0.023	0.040	0.013	0.030	0.011	0.002
5	0.15	–	–	–	–	0.08	–	–	–	–	–	–	–	–	–	–	–
10	0.61	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–	–	–
15	0.79	0.06	0.05	0.96	0.07	0.02	–	–	–	–	0.03	0.002	–	–	–	–	–
20	0.09	0.02	0.08	0.26	0.15	0.01	–	–	–	–	–	–	–	–	–	–	–
25	0.04	–	0.16	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–
30	0.15	–	0.09	0.12	0.11	0.02	–	–	–	–	–	–	–	–	–	–	–
35	0.13	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–
40	0.19	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–
50	0.04	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–	–
Field Trip 01/06/2010																	
0	1.76	0.11	–	–	–	0.03	–	0.07	0.03	0.004	–	0.008	0.027	0.009	0.017	0.008	0.004
5	1.19	0.07	–	–	–	0.01	–	0.11	0.01	0.004	–	0.005	0.026	0.002	0.005	0.010	0.004
11	1.32	0.07	0.46	0.99	0.72	0.01	0.09	–	–	–	–	0.002	0.016	0.003	0.005	–	–
15	0.37	–	0.32	0.64	0.46	0.24	0.07	–	–	–	–	–	–	–	–	–	–
23	0.09	–	2.0	3.97	3.52	0.10	0.2	–	–	–	–	–	–	–	–	–	–
35	0.04	–	0.06	0.07	0.07	–	–	–	–	–	–	–	–	–	–	–	–
40	0.15	0.05	0.19	0.35	0.29	0.04	–	0.04	0.06	0.01	–	–	0.018	0.002	0.002	0.013	0.002
Field Trip 22/07/2010																	
0	0.55	0.05	–	–	–	–	–	0.07	0.02	0.008	0.003	0.008	0.013	0.002	0.004	0.008	0.004
5	0.29	0.09	–	–	–	–	–	0.05	0.01	0.005	0.008	0.004	0.003	0.001	0.004	0.002	0.001
10	0.44	0.04	–	–	–	–	–	0.09	–	–	–	–	0.005	0.002	0.004	0.003	0.003
15	0.10	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–
20	0.00	–	0.35	0.64	0.48	–	–	–	–	–	–	–	–	–	–	–	–
30	0.07	–	0.12	0.22	0.18	–	–	–	–	–	–	–	–	–	–	–	–
40	0.19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Field Trip 13/01/2011																	
0	2.27	0.21	–	–	–	0.03	–	0.2	–	0.017	0.009	0.007	0.04	0.009	0.006	0.010	0.009
5	2.82	0.29	–	–	–	–	–	–	–	0.014	0.014	0.007	0.01	0.004	0.01	0.014	0.012
10	1.49	0.17	–	–	–	0.04	–	0.11	–	0.006	0.007	0.007	0.01	0.003	0.008	0.013	0.003
20	2.16	0.21	–	–	–	0.03	–	0.13	0.02	0.008	0.015	0.008	0.02	0.007	0.018	0.009	0.004
40	1.27	0.12	–	–	–	0.002	–	0.10	–	–	–	0.010	0.004	0.007	0.008	0.007	0.004

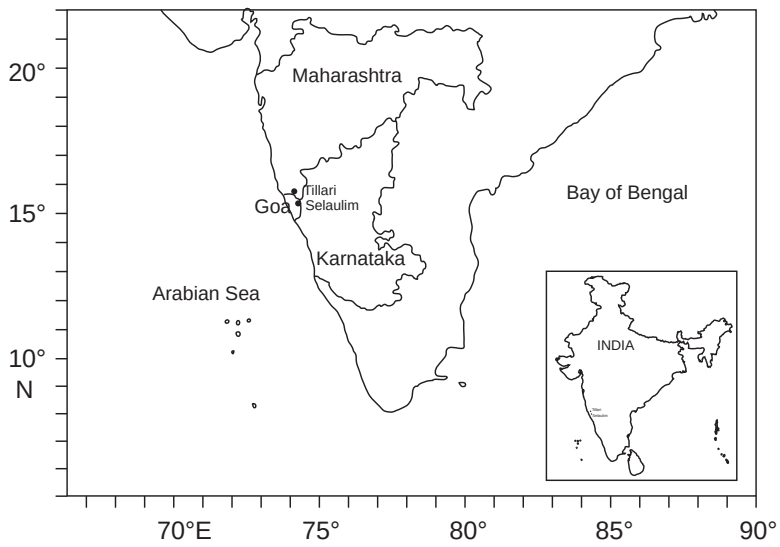

Fig. 1. Sampling locations (Tillari Reservoir and Selaulim Reservoir) situated in central India. Tillari Reservoir is located in the Maharashtra state with storage capacity of 0.45×10^9 m^3 and water depth of ~50 m, while Selaulim Reservoir is located in the Goa state with lower storage capacity (0.23×10^9 m^3) and shallower depth (~20 m). Both these reservoirs were found to turn anoxic during summer.

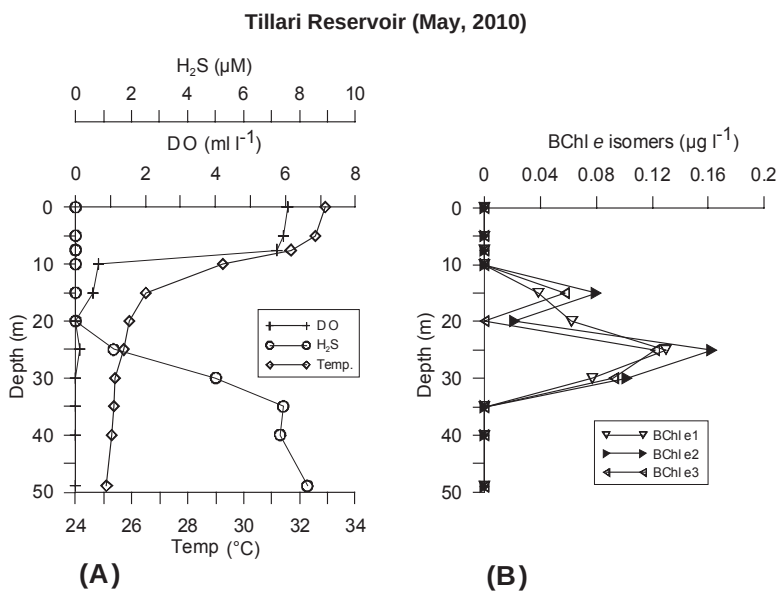

Fig. 2. **(A)** Vertical profiles of temperature (°C), dissolved oxygen (DO; ml l^{-1}) and H_2S (µM) in Tillari Reservoir during 12/05/2010. During summer stratification, water column turned to be sulfidic below 25 m depth. **(B)** Vertical profiles of BChl-*e* isomers (*e*1, *e*2 and *e*3; µg l^{-1}) in Tillari Reservoir.

Discussion Paper | Discussion Paper | Discussion Paper | Discussion Paper |

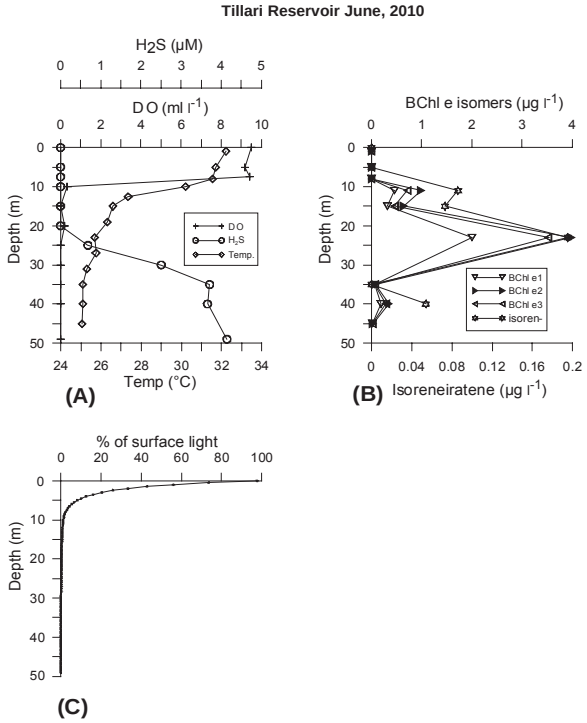

Fig. 3. (A) Vertical profiles of temperature (°C), dissolved oxygen (DO; ml l^{-1}) and H_2S (µM) in Tillari Reservoir during 01/06/2010. **(B)** Vertical profiles of BChl-*e* isomers (*e*1, *e*2 and *e*3; µg l^{-1}) and isoreneiratene (µg l^{-1}) in Tillari Reservoir. **(C)** % of light irradiance calculated as described in Sect. 2.

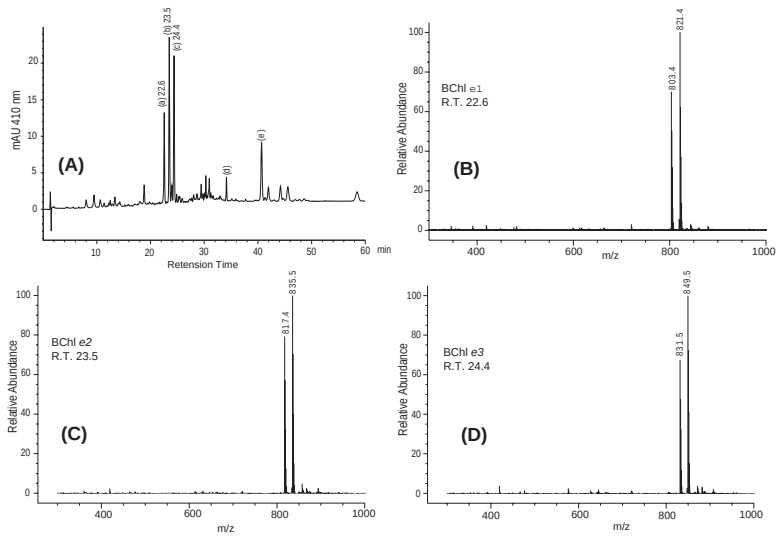

Fig. 4. (A) Representative chromatogram (LC-MS) obtained for suspended particulate matter having bacteriochlorophyll-*e* isomers (Tillari Dam, 01/06/2010, 23 m). Major peaks (a), (b) and (c) represent BChl-*e*1, *e*2 and *e*3 respectively. Peaks (d) and (e) correspond to Chl-*a* and isoreneiratene respectively. **(B)** Confirmative mass spectra (LC-MS) for the peak (a) at 22.6 min. representing BChl-*e*1 isomer (m/z = 821.4, 803.4 ($-H_2O$)). **(C)** Peak (b) at 23.5 min. representing BChl-*e*2 isomer (m/z = 835.5, 817.4 ($-H_2O$)). **(D)** Peak (c) at 24.4 min. representing BChl-*e*3 isomer (m/z = 849.5, 831.5 ($-H_2O$)). R. T. denotes retention time.

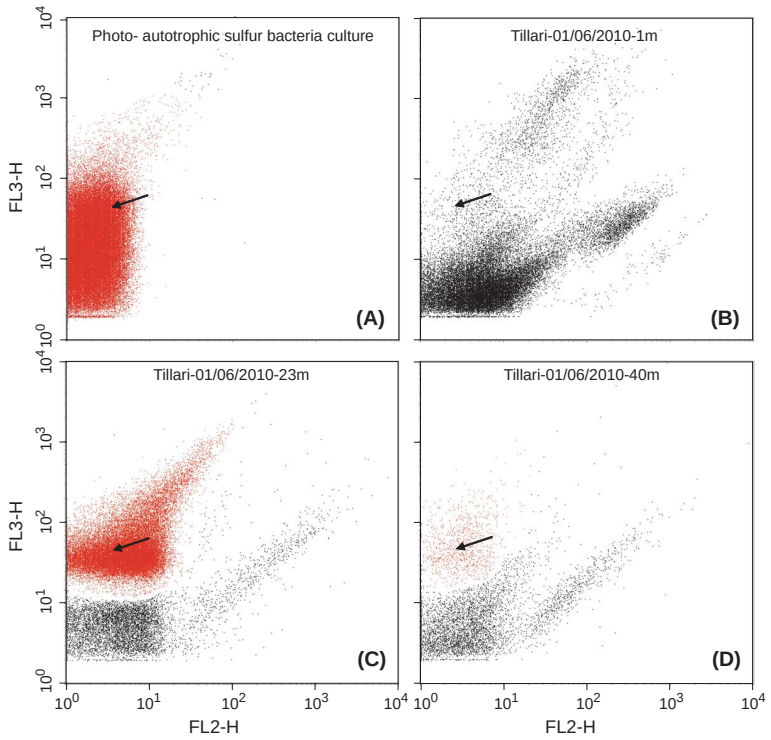

Fig. 5. Flow cytometry (FACS Calibur) analysis of photo-autotrophic sulfur bacteria (PSB) shown in red colour with an arrow. **(A)** Represents mixed culture of PSB, while natural population in Tillari Reservoir at 1 m, 23 m and 40 m are shown in dot plots **(B)**, **(C)** and **(D)** respectively. FL3-H and FL2-H indicates red and orange fluorescence.

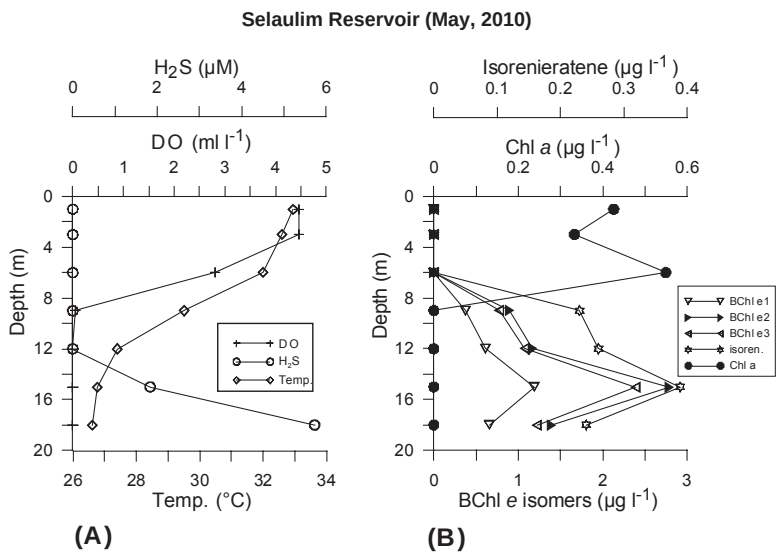

Fig. 6. (A) Vertical profiles of temperature (°C), dissolved oxygen (DO; ml l^{-1}) and H_2S (μM) in Selaulim Reservoir during 21/05/2010. **(B)** Vertical profiles of Chl-*a*, BChl-*e* isomers (*e*1, *e*2 and *e*3) and isoreneiratene (μg l^{-1}) in Selaulim Reservoir.

Discussion Paper | Discussion Paper | Discussion Paper | Discussion Paper |