Oceanologia (2019) 61, 427—444

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.journals.elsevier.com/oceanologia/

ORIGINAL RESEARCH ARTICLE

Spatio-temporal variations in sulfur-oxidizing and sulfate-reducing bacterial activities during upwelling, off south-west coast of India

A. Sam Kamaleson [a], Maria-Judith Gonsalves [a,*], Swatantar Kumar [b], V.K. Jineesh [c], P.A. LokaBharathi [a]

[a] *CSIR-National Institute of Oceanography, Dona Paula, Goa, India*
[b] *International Max Planck Research School of Global Biogeochemical Cycles, Friedrich Schiller University, Jena, Germany*
[c] *Academy of Climate Change, Education and Research, Agricultural University, Kerala, India*

Received 15 October 2018; accepted 13 March 2019
Available online 8 April 2019

KEYWORDS
Sulfate-reduction;
Sulfur-oxidation;
Environmental parameters;
Upwelling phases;
South-west coast of India;
Arabian Sea

Summary The Arabian Sea, off SW India, is becoming more anoxic in recent years. Poor ventilation affects microbial degradation of organic matter in the oxygen minimum zone ($\leq$2.85 ml l^{-1} O_2, $\leq$0.02 μM NO_2) and the anoxic marine zone ($\leq$0.09 ml l^{-1} O_2, $\geq$0.5 μM NO_2). We posit that one of the reasons at the microbial level could be due to a more prominent increase in sulfate-reducing activity (SRA), than sulfur-oxidizing activity (SOA). Hence, the objective was to measure the extent to which SOA can counter the effect of SRA. We, therefore examined these activities along with relevant environmental variables from 2009 to 2011 off Kochi (9.55°N—75.33°E) and Trivandrum (8.26°N—76.50°E), covering the three phases of upwelling. SRA was measured radiometrically using ^{35}S, and SOA by iodometry. Off Kochi, the SOA of the water column increased 6× (194—1151 μM d^{-1}) and SRA 4× (13—54 nM d^{-1}) from phase I to III. Off Trivandrum, the increase in SOA was 1.7× (339—560 μM d^{-1}) and SRA 7× (24—165 nM d^{-1}) contributing to the build-up of reducing/oxidizing conditions. This increase in SOA moderates the effect of increase in SRA. Besides, the average concentrations of dissolved oxygen and nitrite off Trivandrum were 1.80 ± 1.66, 1.48 ± 1.55, 1.93 ± 1.86 ml l^{-1} and 0.14 ± 0.14, 1.69 ± 0.67, 0.34 ± 0.42 μM during the three phases respectively. Hence, it is suggested that the coastal

* Corresponding author at: CSIR-National Institute of Oceanography, Dona Paula, Goa, 403004, India. Tel.: +918322450624; fax: +91(0)832-2450602.
E-mail address: mjudith@nio.org (M.-J. Gonsalves).
Peer review under the responsibility of Institute of Oceanology of the Polish Academy of Sciences.

Production and hosting by Elsevier

https://doi.org/10.1016/j.oceano.2019.03.002

waters examined in this study could probably be between oxygen minimum zone (OMZ) and anoxic minimum zone (AMZ) in patches temporarily. The present paper highlights the interactions between sulfate-reducing and sulfur-oxidizing activities, during upwelling for the first time in these waters. These observations give an important and timely insight into the implications.

1. Introduction

In the North Indian Ocean, off the south-west coast of India, the Arabian Sea water experiences seasonal upwelling during the summer monsoon (May—September). This upwelling phenomenon initiates from Trivandrum (TVM) in the south, and its strength decreases northward (Shetye et al., 1990). The process generally comprises of three different phases, i.e., the initial (May to June: phase I), middle (July to August: phase II) and final (August to September: phase III). During the first phase, primary productivity is at a relatively higher rate triggered by the nutrients brought to the surface. In the second phase, the primary production increases, along with an increase in secondary production. In the third phase, the processes decline, accompanied by plankton degradation by microbes mediating different biogeochemical cycles (Walsh et al., 1999).

While a number of researchers have covered the physical and chemical aspects influencing the biological parameters like primary, secondary and tertiary production (Kumar et al., 2001; Madhupratap et al., 2003; Naqvi, 1991; Shetye et al., 2015), the important aspect of bacterial contribution linking different trophic levels and different parameters have been few. Lately, off TVM and off Kochi, Malik et al. (2015), observed strong relationships between the variables mediated by the microbes.

The maintenance of redox conditions of such coastal systems could depend upon the capacity to oxidize or reduce a certain amount of organic material without significantly changing the redox potential. This net condition could result from several oxidizing and reducing activities prevailing in the system. The relative increase in the sulfate-reducing activity over sulfur-oxidizing activity could be one such important redox cycle that could play a major role in contributing to the lowering of dissolved oxygen and other electron acceptors like nitrate and the "build-up of reducing conditions". It is this accumulation of these reducing conditions/regions coupled with the weak ventilation that could add to the spread of the oxygen minimum zone (OMZ) in space and time. During monsoon/upwelling, intense winds tend to weaken the OMZ in the upper layers by the supply of oxygen through enhanced ventilation which is greater than oxygen consumed by remineralization. However, below thermocline layers (>100 m, Banse et al., 2017), the biological consumption of oxygen exceeds the supply of oxygen by ventilation which causes intensification and expansion of OMZ on a decadal scale (Lachkar et al., 2018). In line with these observations, we have noticed such intensification at the micro-aerophilic to anaerobic level, which particularly revolves around the activities of colourless sulfur-oxidizing (CSOB) and sulfate-reducing bacteria (SRB) and the interactions between them.

Previous studies by Canfield et al. (2010) in the OMZ of Chilean coast have used the metagenomic approach to study the cryptic sulfur cycle. Nevertheless, in our studies, the abundance and activity of pertinent microbial groups were used to understand the spatiotemporal spread in oxidizing/reducing conditions in upwelling waters. However, Banse et al. (2014) stated that 8—12°N of Arabian Sea was outside the suboxic OMZ. Moreover, till 2004, the borders of the OMZ in the Arabian Sea, extended from 18°N to 11°N at a depth of 150—400 m (Banse et al., 2017). More importantly, it has been reported that the Arabian Sea OMZ is weakly dictated by the seasonal cycle of ocean dynamics and the biogeochemistry influenced by the Asian monsoon system of the region. Also, OMZ of this region is "spatially decorrelated" from the coastal upwelling systems where biological productivity is the highest (Resplandy et al., 2012).

However, the participation of microbes and the processes they mediate need to be quantified to appreciate their relative influence from the oxic to the sub-oxic state of the waters. Sulfate-reduction could be an important process in the anaerobic marine environment (Canfield et al., 2005) perhaps even in the OMZ. Organic mineralization by SRA in the marine ecosystem is one of the important terminal degradative processes in low ambient oxygen concentration. It is able to degrade >50% organic matter, accumulating sulfide in the process (Jørgensen and Boetius, 2007). SRB are ubiquitous and are prevalent under both anaerobic and aerobic conditions (Bottrell et al., 1991; Fortin et al., 2002; Gibson, 1990; Winch et al., 2009). Besides, a relatively wide number of sulfate-reducing bacterial genera have been identified from the water column of stratified fjords (Teske et al., 1996). Generally, oxic degradation of organic matter is followed by micro-aerobic to anaerobic breakdown. The predominance of anaerobic bacterial community and their activity over the aerobic counterparts could contribute to the intensification of the reducing conditions in the eastern Arabian Sea (Gonsalves et al., 2011). Besides, SRB and their activity have been known to propagate in regions rich in electron donors like sediments and even in surface layers of euphotic waters (Teske et al., 1996). This is particularly true for upwelling waters where electron donors are supplied both physically due to upwelling and biologically by primary and secondary production.

The colourless sulfur-oxidizing bacteria (CSOB) and their activity could play a major role in oxidizing reduced sulfur and restoring the redox balance in the ecosystem (Jørgensen and Gallardo, 1999; Lavik et al., 2009). SOA could be prominent even in the absence of oxygen, where nitrate could act as an alternate electron acceptor (Fossing et al., 1995). Such interactions between sulfate-reducing and sulfur-oxidizing bacteria have been elucidated but restricted to the sediments of upwelling regions off Namibia, Peru and

Chile using lipid biomarkers (Arning et al., 2008; Canfield, 2001). The present study aims to understand the extent of SRA and SOA in the upwelling waters of the south-west coast of India. Here, the term reducing activity has been used, in the context where the rate of increase in sulfate-reducing activity is more than the rate of increase in sulfur-oxidizing activity during the three phases of the study.

We posit that one of the explanations for the restoration of redox balance could be a higher "increase" in sulfur-oxidizing activity (SOA) when compared to "increase" in sulfate-reducing activity (SRA). However, a significantly large imbalance in which SRA exceeds SOA could lead to the spread of reducing conditions in time and space. Hence, the activity and the distribution patterns of bacteria responsible for these processes were examined along the transects off Kochi and Trivandrum during the initial, middle and final phases of upwelling. The present paper highlights the interactions between sulfate-reducing and sulfur-oxidizing bacterial abundance and their activities, along with the related environmental parameters during upwelling.

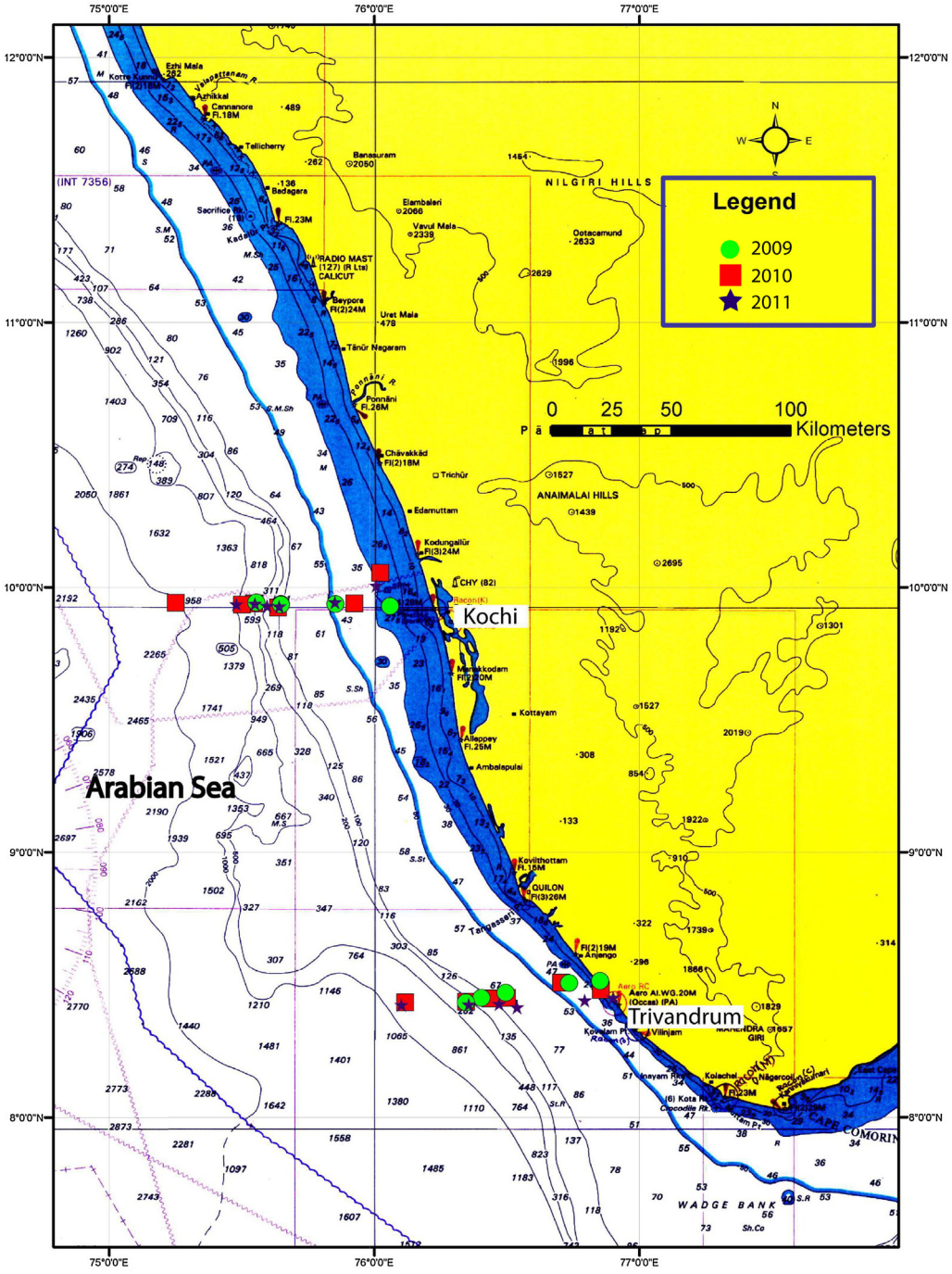

Figure 1 Details of sampling locations off Kochi and Trivandrum, southwest coast of India during phase I (2009), phase II (2010) and phase III (2011) of upwelling.

Table 1 Details of sampling depths in the southwest coast of India during phase I (2009), phase II (2010) and phase III (2011) of upwelling.

Station no.	Water depth [m]	Sampling depth [m] Kochi and Trivandrum
1	30	5, 15, 25
2	50[a]	5, 10, 25, 45
3	100	5, 20, 60, 80, 90
4	200	5, 20, 60, 80, 150, 175
5	500[b]	5, 20, 60, 80, 150, 300, 450

Total number of samples, *n*: Phase I (2009) = 25; Phase II (2010) = 28; Phase III (2011) = 32.
[a] No sampling during Phase II.
[b] No sampling during Phase I.

2. Material and methods

2.1. Description of the study sites

Water samples were collected onboard FORV *Sagar Sampada*, during cruises #267 (2009), #276 (2010), #289 (2011), along the transect off Trivandrum and Kochi in the south-eastern Arabian Sea at 8.26°N–76.50°E & 9.55°N–75.33°E (Fig. 1), during the three phases of upwelling – end-May to end-June 2009, initial phase (phase I), July to mid-August 2010, middle phase (phase II) and mid-August to mid-September 2011, final phase (phase III). Hereafter, these samplings would be referred to as phase I, II and III respectively. Samples were collected at different depths from six different stations in each transect (Table 1).

2.2. Sample collection

Water samples were collected using 10 L Niskin bottles fixed to CTD-rosette system. Bottles were rinsed twice with sample water before sub-sampling for parameters like dissolved oxygen (DO), nutrients (nitrate, nitrite, phosphate and silicate), chlorophyll *a* (Chl *a*), phaeopigments, total counts (TC) and total viable counts (TVC) of bacteria, sulfate-reducing bacteria (SRB) and colourless sulfur-oxidizing bacteria (CSOB). Experiments were conducted for SRA and SOA and were incubated onboard.

2.3. Chemical parameters

Dissolved oxygen was measured by Winkler's titrimetric method (Strickland and Parsons, 1977). Samples were collected in 125 ml glass stoppered bottles without air bubbles and were immediately fixed with Winkler's reagents. Fixed samples were stored in the dark for further analyses. DO samples were analyzed onboard using a dosimeter (Metrohms785 DMP Titrino). Analyses of nutrients were carried out onboard using a SKALAR auto analyzer (Wurl, 2009). Chlorophyll *a* (Chl *a*) concentrations were determined (Yentsch and Menzel, 1963) fluorometrically (Turner Designs, USA). Samples were filtered on GF/F filters (0.7 μm) and the extraction was done by adding 10 ml of 90% acetone to the filter and incubating for 24 h in the dark under refrigerated condition. For phaeopigments, the Chl *a*, samples were acidified with 10% HCl just before fluorometric measurement (Yentsch and Menzel, 1963). Ammonium was estimated manually by spectrophotometric method (Grasshoff et al., 1983).

2.4. Bacteriological parameters

The TC of bacteria was determined by acridine orange direct count (AODC) method using epifluorescence microscopy and expressed as numbers per litre. Aliquots of 5 ml water sample in triplicate were each fixed with 250 μL of buffered formalin (2% final concentration) as described by Hobbie et al. (1977). TVC was done quantitatively as outlined by Kogure et al. (1984). In brief, the yeast extract in the incubating medium allows for an increase in size but the cocktail of antibiotics used prevented the cell division, thus enabling the enumeration of enlarged cells. The fixed water samples of TVC were stained for 5 min with acridine orange (final concentration 2%) and filtered through 0.22 μm Millipore black nucleopore membrane filters. The bacterial cells were counted by epifluorescence microscope (Nikon Epifluorescence Microscope, Model A 80i) and expressed as numbers per litre.

SRB were enumerated using the most probable number (MPN) method, with the liquid media supplemented with 0.5 mM lactate and 0.5 mM acetate as carbon sources (Hatchikian's medium (1972) modified by LokaBharathi and Chandramohan (1990)). The samples were inoculated in screw-capped tubes and incubated at 27 ± 2°C and were enumerated after 15–20 days.

Modified Leiske's medium with 10% of the recommended thiosulfate concentration (0.5 g l^{-1}) was used for the enumeration of CSOB by MPN technique (LokaBharathi, 1989 and references therein). The samples were incubated at 27 ± 2°C for 5–7 days and the numbers were expressed as cells l^{-1}.

MPN method used as the target organisms were less in numbers and difficult to grow on medium containing agar. This method also helps to measure the number and activity simultaneously. Though the method selected is used for fast-growing bacteria on liquid medium, it is also useful for comparing trends in the distribution of numbers and activity across samples (LokaBharathi et al., 1988).

2.5. Sulfate-reducing activity

SRA was measured in the terms of rate (SRR) expressed in nM d^{-1}. The method was described earlier by King (2001) and was adopted for coastal waters by Lavik et al. (2009). Radioactive sodium sulfate 300 μl ($^{35}SO_4^{-2}$, specific activity, 37 kBq from BARC, Mumbai) was injected into a known volume of sample in gas-tight vials and the activity was arrested at the end of 72 h by adding 5 ml (5% w/v) zinc acetate and maintained at 4°C until further analysis. At each station, zinc acetate was added to each tube before the tracer addition to determine a blank. Radioactivity was measured using a liquid scintillation counter (Perkin Elmer Wallace 1409 DSA). SRA was calculated using the equation:

$$\mathrm{SRA(dpm)} = \left(\frac{H_2{}^{35}S}{{}^{35}SO_4{}^{2-}}\right) \times {}^{32}SO_4{}^{2-} \times \frac{IDF}{T},$$

where SRA is the sulfate-reducing activity, $H_2{}^{35}S$ the radioactivity of reduced sulfur in DPM (disintegration per minute), $^{35}SO_4{}^{2-}$ the radioactivity of sulfate at the beginning of incubation, $^{32}SO_4{}^{2-}$ the sea water sulfate concentration in mM $SO_4{}^{2-}$, IDF the isotopic discrimination factor = 1.06, and T is the time of incubation in hours.

The SRA was calculated from the amount of radioactive sulfate used and was expressed in nM $SO_4{}^{2-}$ d^{-1}. The SRB medium contained both lactate and acetate in order to assess the SRA at the expense of both the major substrates.

2.6. Sulfur-oxidizing activity

The water samples were tested for their ability to oxidize thiosulfate by measuring the residual concentration of thiosulfate, iodimetrically (Hansen, 1973). To 200 ml of sample, 50 ml of diluted sulfuric acid (3 N), and 25 ml of 0.0125 N iodine solution were added and titrated immediately with standardized 0.0125 N $Na_2S_2O_3{\cdot}5H_2O$ until most of the iodine was consumed. A few drops of freshly prepared starch solution (1%) was added and titrated until the blue colour disappeared. Blanks were estimated as mentioned below. The method was suitably adopted to estimate MPN and to measure the sulfur-oxidizing activity. The experimental tubes were incubated at $27 \pm 2^{\circ}C$ for 5–7 days. Uptake of thiosulfate was expressed as $\mu M\ S_2O_3\ d^{-1}$.

Titre value of sea water blank (Avg): A
Titre value of un-inoculated media (Avg): B
Average titre value of sample: S

$$\frac{A-B\times 1400}{\text{Volume of sea water used for titration (100 ml)}} = B1\ (\text{thiosulfate measured in medium}),$$

$$\frac{A-S\times 1400}{\text{Volume of sea water used for titration (100 ml)}} = S1\ (\text{thiosulfate measured in sample}),$$

where 1400 = mol. wt. of S_2O_3 ($112 \times 0.0125\ N \times 1000$),

$$B1-S1 = D\ (\text{amount of thiosulfate utilized [mg]}) = D/112\ (\text{mol. wt. of } S_2O_3) \times 1000\ (\text{mg to } \mu g)/\text{incubation in days} = D\ \mu M d^{-1}.$$

The rates of these processes are generally measured in nM for sulfate reduction (Albert et al., 1995) and μM for thiosulfate oxidation (Tuttle and Jannasch, 1976; Visscher et al., 1992). This difference in the activity is attributable to ecological and technical reasons. While the product of the former (sulfide) is environmentally toxic to fauna even in low concentrations from nM to μM, the latter, is relatively benign. Therefore, there is a 3–4 order difference between the two activities. Technically the methodology for sulfate reduction based on radiometry is much more sensitive to lower concentrations than the iodometric method for thiosulfate oxidation.

It is, therefore, suggested that the spread of reducing activity may not be directly based on the stoichiometry but on the 'actual changes in the rates' of these two activities over the three different phases of upwelling. It should be noted that the main focus is on measuring the "relative increase" in these activities over the three phases.

2.7. Statistical analysis

Biological data was $\log(x+1)$ transformed for statistical analysis. Correlations between biological and environmental parameters were determined using Statistica version 6.0. One-way analysis of variance (ANOVA) was performed on data to find the significant variation between SRA, SOA from phase I to phase III. The analysis was carried out using Statistica 6.0 software package.

3. Results

Comparison of transects, parameter wise, off Kochi and TVM, was performed to reveal how microbial variables especially SRA/SRB and SOA/CSOB responded to differences in the physical, chemical and biological forcings. The vertical variations in these parameters were more off Kochi than off TVM.

3.1. Physico-chemical parameters

Off Kochi, from phase I to phase III, the average values of physico-chemical parameters like temperature (23.58–20.31°C), pH (7.97–7.56), salinity (35.05–34.77) and DO concentrations (2.03–1.82 ml l^{-1}) showed decreasing trends (Table 2) with a dip in the DO concentration in the second phase of 1.75 ml l^{-1}. The average values of parameters for the three different phases showed decreasing trends. This decrease is not statistically significant. However, the water column averages of some chemical parameters like nitrate, nitrite and phosphate showed little variation over the study period. Other chemical parameters like silicate concentrations decreased from 7.64 to 5.08 μM with a dip in the second phase (0.32 μM), and ammonium concentration showed a definite increase from 0.07 to 1.51 μM (Table 2, Fig. 2a).

Off TVM, the average values of the parameters from phase I to phase III, like temperature (21.56–20.69°C), pH (7.98–7.50) and salinity (34.97–34.84), also showed decreasing trends with little or no statistical significance. The nutrient distribution was different off TVM, from that off Kochi. Off TVM, nitrite, phosphate, and silicate did not show any definite trend, whereas nitrate and ammonium increased 1.3× (15.55–20.22 μM) and ~7× (0.19–1.31 μM) respectively (Fig. 2b). In the first phase, the range in the concentration of phosphate (0.18–2.96 μM), silicate (0.47–28.15 μM), and nitrate (0.02–37.78 μM) levels were higher off TVM than off Kochi.

Off Kochi, the SRA was not depth dependent, while off TVM, SRA decreased with depth, i.e. about 10% of the variation in SRA was negatively influenced by depth ($r = -0.315$; $r^2 \times 100 = 10\%$) in phase I and 13% in phase III. Off Kochi and TVM the SRB was depth dependent in phase I and phase III. However, the temperature had a positive influence of 36% on the distribution of SRB ($p < 0.05$) in phase I off Kochi.

Off TVM, nutrients affect the distribution of CSOB in phase I, phase III and that of SRB in phase II. Relationship of SRB with

Table 2 Range (bold), average and standard deviation (±) of physico-chemical and biological parameters during the three phases of upwelling (2009–2011).

Parameters	Phase I		Phase II		Phase III	
	Kochi	Trivandrum	Kochi	Trivandrum	Kochi	Trivandrum
Temperature (°C)	[a]**13.46–28.88**	**10.90–29.18**	**11.30–26.90**	**11.25–28.30**	**7.26–27.03**	**7.13–28.04**
	[b]23.58 ± 4.97	21.56 ± 4.94	23.06 ± 4.03	20.07 ± 5.80	20.31 ± 5.79	20.69 ± 6.17
Salinity (%)	**34.88–35.61**	**34.80–35.30**	**34.30–35.60**	**34.30–35.20**	**33.53–35.21**	**34.15–35.15**
	35.05 ± 0.20	34.97 ± 0.13	35.07 ± 0.29	34.97 ± 0.21	34.77 ± 0.43	34.84 ± 0.29
pH	**7.68–8.30**	**7.69–8.50**	**7.76–7.93**	**7.70–8.26**	**7.32–7.85**	**7.25–7.78**
	7.97 ± 0.23	7.98 ± 0.24	7.92 ± 0.23	7.90 ± 0.17	7.56 ± 0.18	7.50 ± 0.18
Phosphate (μM)	**0.16–2.42**	**0.18–2.96**	**0.35–4.51**	**0.85–1.56**	**0.23–2.16**	**0.30–5.16**
	1.44 ± 0.74	1.21 ± 0.81	1.49 ± 1.57	1.06 ± 0.25	1.25 ± 0.58	1.70 ± 0.94
Silicate (μM)	**0.29–19.45**	**0.47–28.15**	**ndl–1.74**	**0.07–4.75**	**1.07–12.40**	**0.71–18.76**
	7.64 ± 5.71	7.75 ± 7.48	0.32 ± 0.50	1.65 ± 1.12	5.08 ± 2.96	5.80 ± 4.30
Nitrate (μM)	**0.64–33.17**	**0.02–37.78**	**ndl–15.40**	**0.51–17.39**	**0.06–36.50**	**ndl–40.84**
	18.20 ± 11.26	15.55 ± 12.62	11.36 ± 7.37	12.56 ± 6.14	17.55 ± 13.37	20.22 ± 11.99
Nitrite (μM)	**0.04–1.96**	**ndl–0.68**	**0.13–7.0**	**1.24–4.25**	**ndl–2.15**	**ndl–1.39**
	0.34 ± 0.51	0.14 ± 0.14	1.92 ± 4.47	1.69 ± 0.67	0.28 ± 0.46	0.34 ± 0.42
Ammonium (μM)	**ndl–0.14**	**ndl–1.23**	**0.03–4.59**	**0.13–4.59**	**0.63–4.79**	**0.57–2.75**
	0.07 ± 0.06	0.19 ± 0.27	0.47 ± 1.06	0.79 ± 1.25	1.51 ± 0.80	1.31 ± 0.55
Chl *a* (μg l^{-1})	**ndl–2.18**	**ndl–1.04**	**0.35–17.45**	**ndl–3.49**	**0.07–15.31**	**0.02–1.90**
	0.67 ± 0.70	0.34 ± 0.33	4.97 ± 6.28	0.63 ± 1.03	1.96 ± 3.19	0.65 ± 0.58
Phaeopig* (μg l^{-1})	**ndl–0.76**	**ndl–0.87**	**0.80–2.17**	**0.02–2.67**	**ndl–10.72**	**ndl–2.98**
	0.28 ± 0.22	0.23 ± 0.21	1.29 ± 0.44	0.49 ± 0.68	1.06 ± 2.24	0.57 ± 0.58
Phaeopig/Chl *a*	**0.01–2.50**	**0.15–4.64**	**0.05–3.16**	**0.44–9.50**	**0.01–4.66**	**0.12–9.17**
	1.01 ± 0.80	1.38 ± 1.23	2.67 ± 9.25	2.65 ± 2.52	1.23 ± 1.36	1.85 ± 2.08
DO (ml l^{-1})	**0.29–4.88**	**0.03–4.40**	**0.54–4.50**	**0.23–4.57**	**0.40–5.24**	**0.33–5.17**
	2.03 ± 1.70	1.80 ± 1.66	1.75 ± 1.73	1.48 ± 1.55	1.82 ± 1.73	1.93 ± 1.86
TC × 10^8 l^{-1}	**8.21–8.73**	**8.32–8.91**	**8.11–10.58**	**8.83–10.17**	**0.50–7.12**	**0.94–9.56**
	8.39 ± 0.13	8.59 ± 0.14	9.48 ± 0.77	9.09 ± 0.27	3.20 ± 1.67	4.36 ± 2.13
TVC × 10^7 l^{-1}	**7.82–8.18**	**7.70–8.0**	**7.84–9.07**	**7.09–8.83**	**0.23–4.43**	**0.21–6.05**
	7.97 ± 0.11	7.84 ± 0.09	8.50 ± 0.32	8.44 ± 0.41	1.72 ± 1.03	1.98 ± 1.51
SRA (nM d^{-1})	**1.37–54.98**	**5.87–90.65**	**21.08–28.79**	**19.24–28.43**	**12.76–290.15**	**0.71–398.35**
	13.72 ± 14.55	23.90 ± 30.83	26.19 ± 2.15	22.89 ± 2.32	54.04 ± 71.90	165.17 ± 155.45
SRB × 10^4 l^{-1}	**0.05–0.91**	**0.18–1.50**	**0.04–2.20**	**0.20–4.0**	**0.01–0.90**	**0.01–0.96**
	0.46 ± 0.31	0.52 ± 0.34	0.33 ± 0.51	1.40 ± 1.17	0.25 ± 0.27	0.36 ± 0.30
SOA (μM d^{-1})	**18.74–568.62**	**9.39–601.71**	**33.85–729.58**	**3.76–793.51**	**348.46–1747.44**	**6.45–1728.10**
	194.25 ± 205.84	339.29 ± 195.37	360.19 ± 213.43	215.10 ± 232.67	1151.06 ± 365.16	560.93 ± 388.60
CSOB × 10^6 l^{-1}	**0.26–1.56**	**0.28–1.71**	**0.12–1.08**	**0.06–0.66**	**0.01–0.20**	**0.01–0.37**
	0.72 ± 0.45	0.83 ± 0.34	0.46 ± 0.23	0.34 ± 0.19	0.10 ± 0.05	0.15 ± 0.09

* *Note*: ndl, non-detectable limits; DO, dissolved oxygen; Chl *a*, chlorophyll *a*; phaeopig, phaeopigment; TC, total counts; TVC, total viable counts; SRB, sulfate-reducing bacteria; SRA, sulfate-reducing activity; SOA, sulfur-oxidizing activity; CSOB, colourless sulfur-oxidizing bacteria.

[a] Range.

[b] Average and standard deviation.

ammonium evolved from negative ($r = -0.394$, $p < 0.05$) to positive ($r = 0.424$, $p < 0.05$) from phase I to phase III and synchronized with an increase in phaeopigment from 0.23 μg l^{-1} in phase I to 0.57 μg l^{-1} in phase III. The SOA negatively influenced ambient phosphate (26%, $p < 0.01$), silicate (24%, $p < 0.01$) and nitrate concentrations (18%, $p < 0.05$) in phase I. In phase II, SOA positively correlated with nitrite ($r = 0.430$, $p < 0.05$) (Table 3).

Both groups of bacteria were influenced differently with phosphate. In phase I, off TVM, SOA related negatively with phosphate ($r = -0.506$, $p < 0.01$), while in phase II, SRB related positively ($r = 0.615$, $p < 0.01$). Silicate was influenced positively by SRB ($r = 0.446$, $p < 0.05$) in phase II and negatively by SOA ($r = -0.490$, $p < 0.01$) in phase I. However, SRB showed positive relation with phosphate ($r = 0.615$, $p < 0.01$) and silicate ($r = 0.446$, $p < 0.05$) in phase II.

3.2. Biological parameters

Off Kochi, the average Chl *a* values increased from 0.67 to 1.96 μg l^{-1} from phase I to phase III. High Chl *a* values

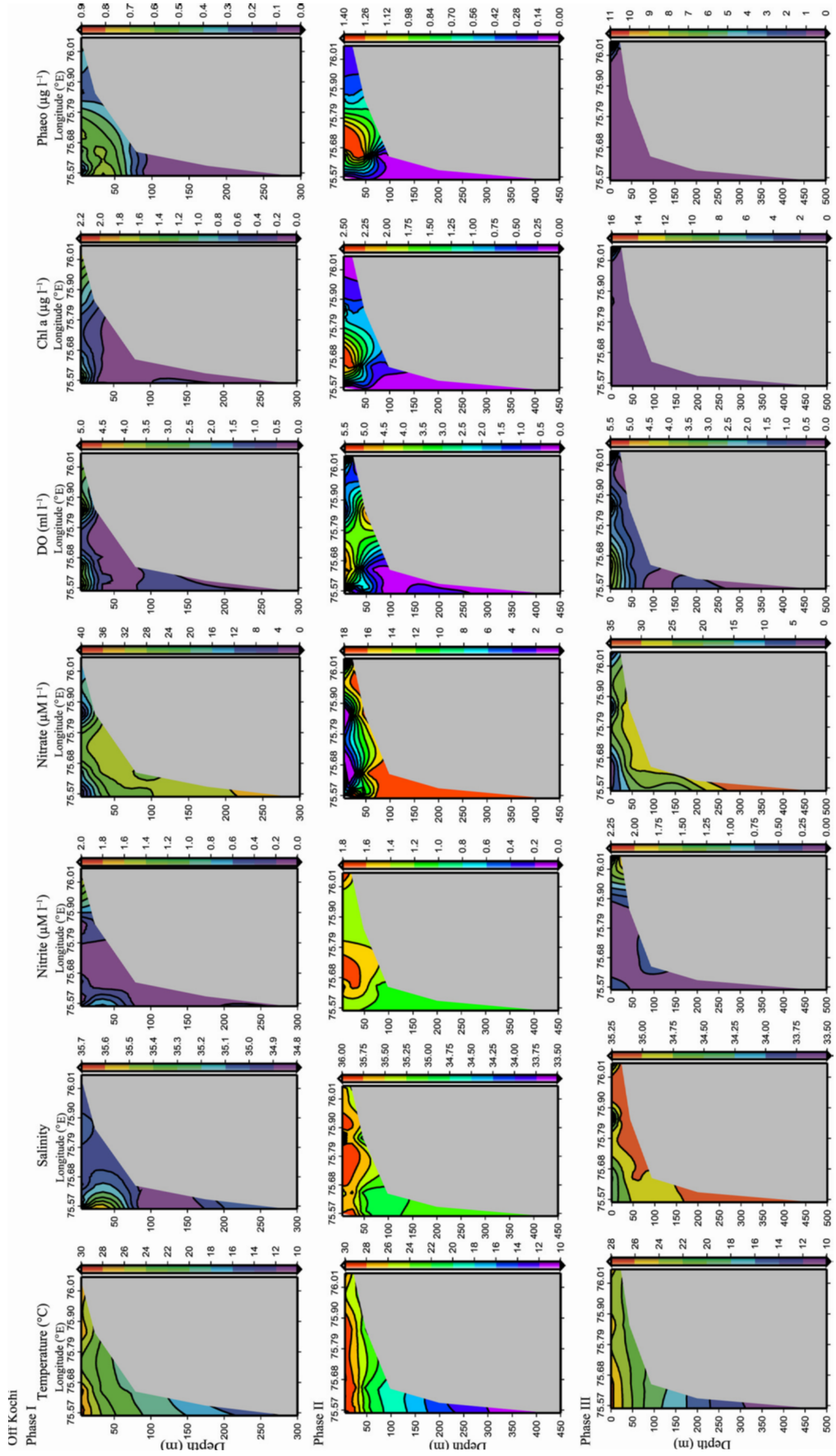

Figure 2 Distributory pattern of environmental parameters off (a) Kochi and (b) Trivandrum (TVM), showing depth wise variation on *y*-axis and distance from the shore on *x*-axis.

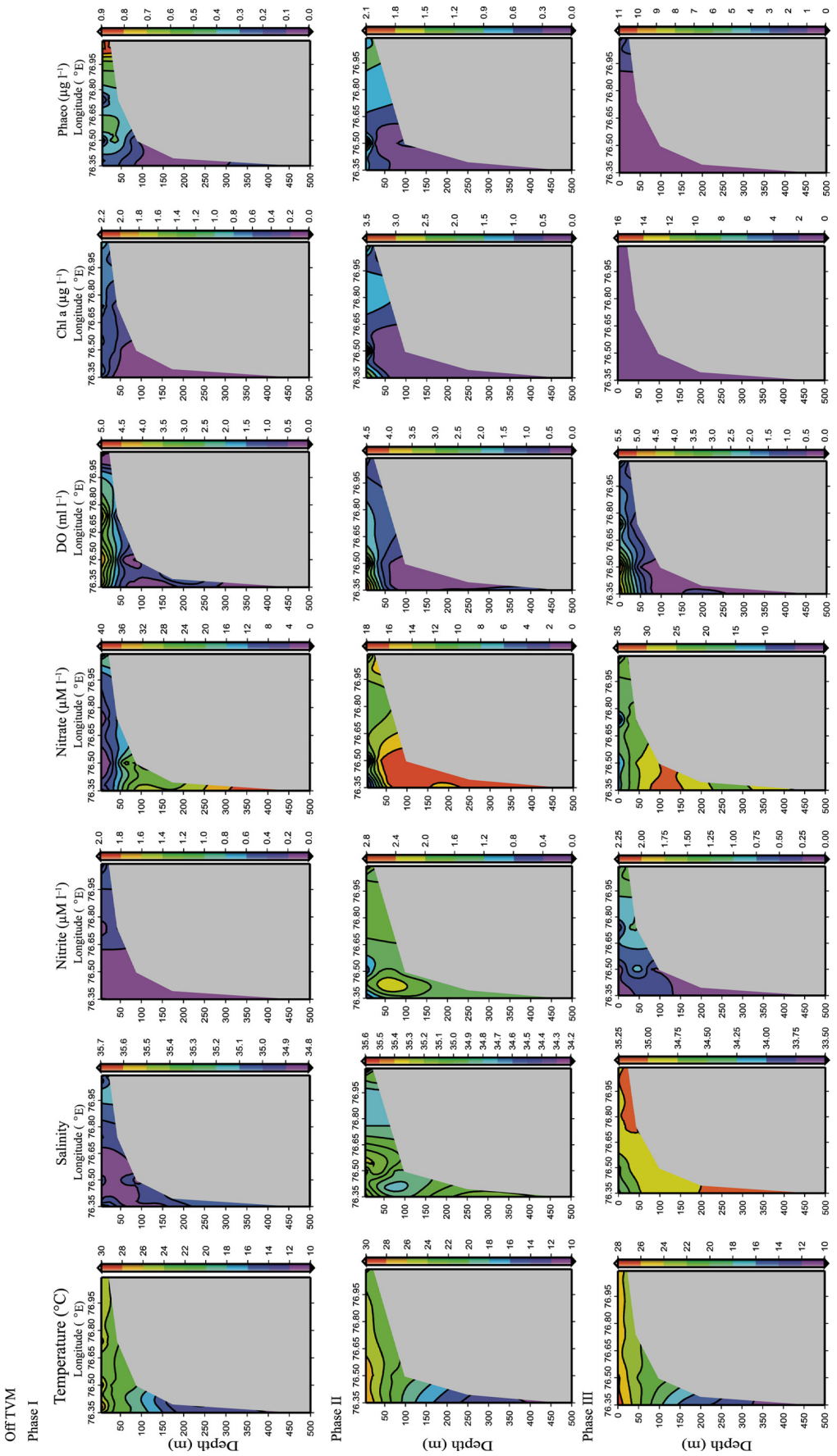

Figure 2 (*Continued*).

Table 3 Significant correlation of sulfate-reducing bacteria (SRB), sulfate-reducing activity (SRA), colourless sulfur-oxidizing bacteria (CSOB) and sulfur-oxidizing activity (SOA) with various environmental parameters.

Parameters	Kochi			Trivandrum		
	Phase I	Phase II	Phase III	Phase I	Phase II	Phase III
Depth	SRB (−0.545*)	CSOB (0.589**)		SOA (0.524*)	SRB (0.782***)	SRA (−0.359*)
Temperature	SRB (0.593*)	–	SRB (0.396*)	SRB (0.394*), SRA (0.396*), SOA (0.524*)	–	–
Nutrients	–	CSOB (NO_2^- 0.530*)	–	CSOB (NH_4^+ −0.394*), SOA (PO_4^{3-} −0.506**, SiO_4^{4-} −0.490**, NO_3^- −0.420*)	SRB (PO_4^{3-} 0.615**, SiO_4^{4-} 0.446*, NO_3^- 0.435*), SOA (NO_2^- 0.430*)	CSOB (NH_4^+ 0.424*, NO_2^- 0.417*), SOA (NO_3^- 0.364*)
Chlorophyll *a*	–	–	SOA (−0.523*)	SRB (0.572**), SRA (0.595**)	SRB (−0.451*)	SRA (0.382*)
Phaeopigments	–	CSOB (0.538*)	SOA (−0.549*)	SRB (0.539**), SRA (0.652***)	SRB (−0.442*)	–

Note: Values in parenthesis indicate *r* values (correlation coefficient). Chl *a*, chlorophyll *a*; phaeo, phaeopigments; PO_4^{3-}, phosphate; SiO_4^{4-}, silicate; NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

(4.97 μg l^{-1}) off Kochi in phase II was matched by low silicate (non-detectable levels (ndl) to 1.74 μM) and nitrate concentrations (ndl to 15.4 μM). Consequently, like Chl *a*, phaeopigments and phaeo/Chl *a* ratio also showed increasing trends over the phases from 0.28 to 1.06 μg l^{-1} and 1.01 to 1.23 μg l^{-1} respectively (Table 2).

Off TVM, the average Chl *a* increased from 0.34 μg l^{-1} in phase I, to 0.63 μg l^{-1} in phase II, and to 0.65 μg l^{-1} in phase III (Table 2). Like the transect off Kochi, the average phaeopigment concentrations off TVM showed a definite increasing trend from 0.23 μg l^{-1} to 0.57 μg l^{-1} from phase I to phase III. The phaeopigment/Chl *a* ratio ranged from 0.15–4.64 in phase I, 0.44–9.50 in phase II and 0.12–9.17 in phase III. The whole water column average of phaeo-pigment/Chl *a* ratio was 1.38 in phase I, 2.65 in phase II and 1.85 at phase III.

Off TVM, in phase I, SRB and SRA varied positively with Chl *a* ($r = 0.572$, $r = 0.595$, $p < 0.01$ respectively). In phase II, SRB varied negatively with Chl *a* ($r = -0.451$, $p < 0.05$) and in phase III, it was not influenced. However, in phase III, SRA varied positively with Chl *a* ($r = 0.382$, $p < 0.05$). In phase I, SRB and SRA varied positively with phaeopigment ($r = 0.539$, $p < 0.01$, $r = 0.652$, $p < 0.001$ respectively). In phase II, SRB varied negatively with phaeopigment ($r = -0.442$, $p < 0.05$), whereas in phase III, no such relationship was observed with SRB and SRA. Such relationships were not observed off Kochi (Table 3).

3.3. Microbiological parameters — abundance

Off Kochi, total bacterial counts were in the order of 10^8 l^{-1} and did not show significant variation over the period examined. Similarly, there was no appreciable change in the direct viable count which was one order less. Off TVM, the total bacterial abundance and viability was 4.36–8.59 × 10^8 l^{-1} and 1.98–7.84 × 10^7 l^{-1} respectively.

Off Kochi, the average SRB population showed low variation from 0.25 × 10^4 l^{-1}–0.46 × 10^4 l^{-1}. Off TVM, the range in SRB abundance was from 10^2 to 10^4 l^{-1}. Generally, higher SRB population was observed in the upwelling waters off TVM. Off Kochi, CSOB did not show any logarithmic variation and was in the range of 0.1 × 10^6 l^{-1} to 0.72 × 10^6 l^{-1}. Off TVM, CSOB populations varied from 0.15 × 10^6 l^{-1} to 0.83 × 10^6 l^{-1} (Fig. 3a and b, Table 2).

Spearman's correlation analysis showed TC to correlate with environmental parameters at phase I only off TVM (depth: $r = -0.473$, $p < 0.05$, temperature: $r = 0.631$, phosphate: $r = -0.664$, silicate: $r = 0.609$, nitrate: $r = -0.696$, ammonium: $r = 0.606$, Chl *a*: $r = 0.577$, phaeo/Chl *a*: $r = -0.502$, $p < 0.001$).

3.4. Microbiological parameter — activity

There was a general increase in reduction processes over the phases. Off Kochi, the average SRA increased from 13.72 nM d^{-1} in phase I, to 26.19 nM d^{-1} in phase II, and 54.04 nM d^{-1} during phase III. Off TVM, the average SRA increased from 23.90 to 165 nM d^{-1} from phase I to phase III, with a lower rate of 22.89 nM d^{-1} in phase II.

Off Kochi, SOA values were 194, 360, 1151 μM d^{-1}, for the three phases respectively. Comparatively, off TVM low SOA of 339, 215 and 560 μM d^{-1} was noticed during the three phases respectively.

Off Kochi, the average water column SRA increased 4 times, and SOA 6× from phase I to III. The increase in offshore SRA was 5.9× (10.09 nM d^{-1} to 59.58 nM d^{-1}) over these phases. Off TVM, the increase in column SRA was 7× (24 nM d^{-1} to 165 nM d^{-1}) and SOA was 1.7×. The increase in offshore SRA was 9× (13.94 nM d^{-1} to 126.65 nM d^{-1}) over the phases (Fig. 4).

Analysis of variance (ANOVA) of the whole column of SRA and SOA off Kochi and TVM further support our observation on the differences in the 2 processes between the transects. The increase in SRA from phase I to phase III is more significant off TVM ($p < 0.001$) than Kochi ($p < 0.03$). SOA, on the other hand, is more significant off Kochi ($p < 0.001$) than off TVM ($p < 0.04$) (Table 4).

4. Discussion

The results are discussed in the context of physicochemical parameters off Kochi, and TVM, followed by biological and microbial parameters. The interaction of geochemical variables of sulfur bacteria and their activities highlight for the first time, the contribution of this group to the ecology of the upwelling system in these waters.

The physical oceanographic processes influence the input of nutrients to the nutrient-impoverished waters of Arabian Sea (Goes et al., 2005; Wiggert et al., 2002) thus making it one of the most productive areas (Madhupratap et al., 1996). The decreasing trends of temperature, pH, DO in both the transects could be attributed to the different phases of upwelling which were associated with the different stages of monsoon. The upwelling is discernible by the presence of cooler more saline and denser water containing less DO than the normal waters of the surface layers (Fig. 2a and b).

The differences in the parameters off the two transects could be attributed to the difference in the upwelling intensity due to the differences in the strength of physical forcing of upwelling (Shetye et al., 1990).

Off Kochi, the increase in Chl *a* and phaeopigments at phase II has been noticed (Fig. 2a). The increase in the range of phaeopigments, and phaeo/Chl *a* ratio from phase I to

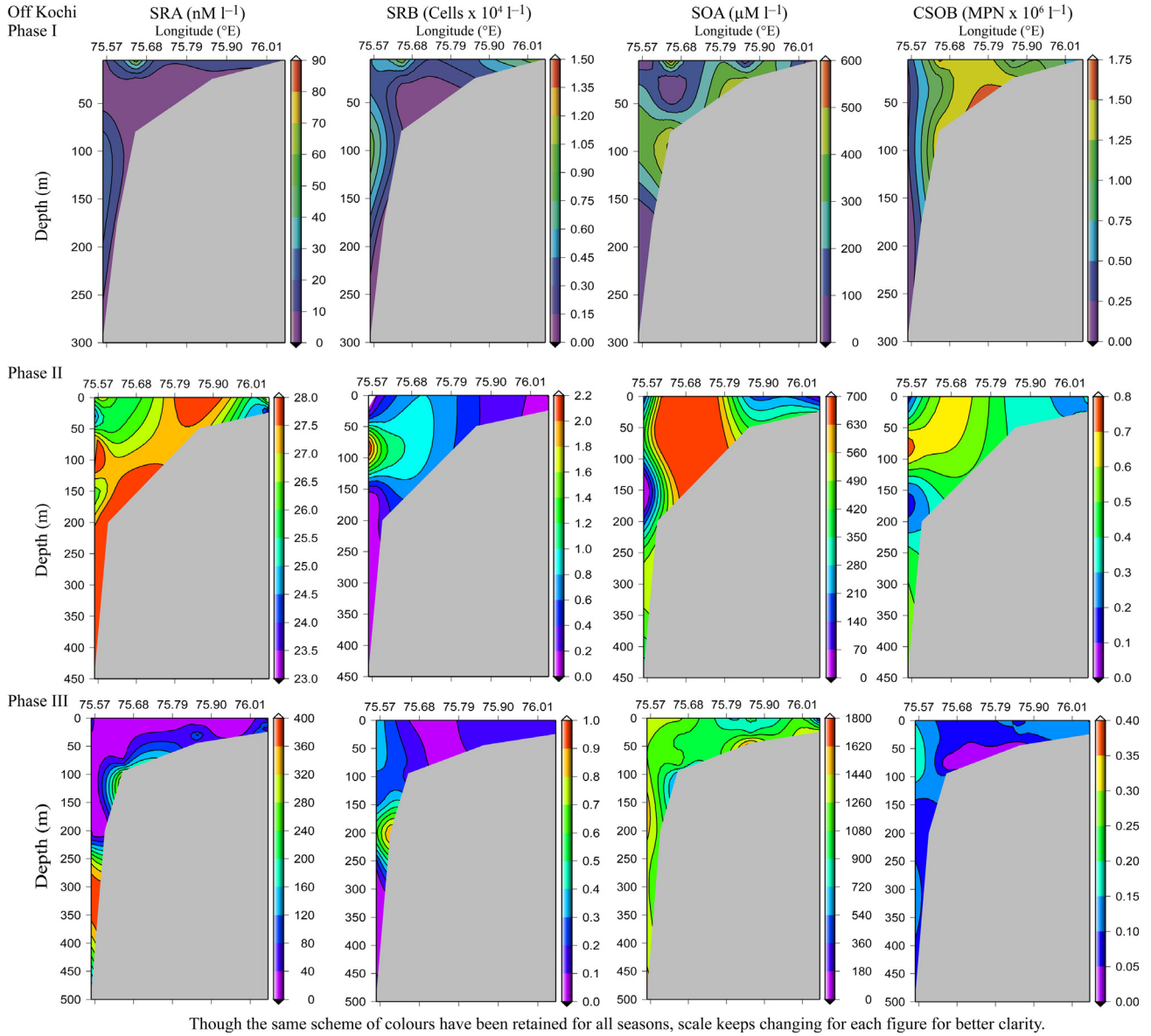

Figure 3 Distributory pattern of sulfate-reducing activity (SRA), sulfate-reducing bacteria (SRB), sulfur-oxidizing activity (SOA) and colourless sulfur-oxidizing bacteria (CSOB) off (a) Kochi and (b) Trivandrum (TVM) in southwest coast of India during phase I (2009), phase II (2010) and phase III (2011).

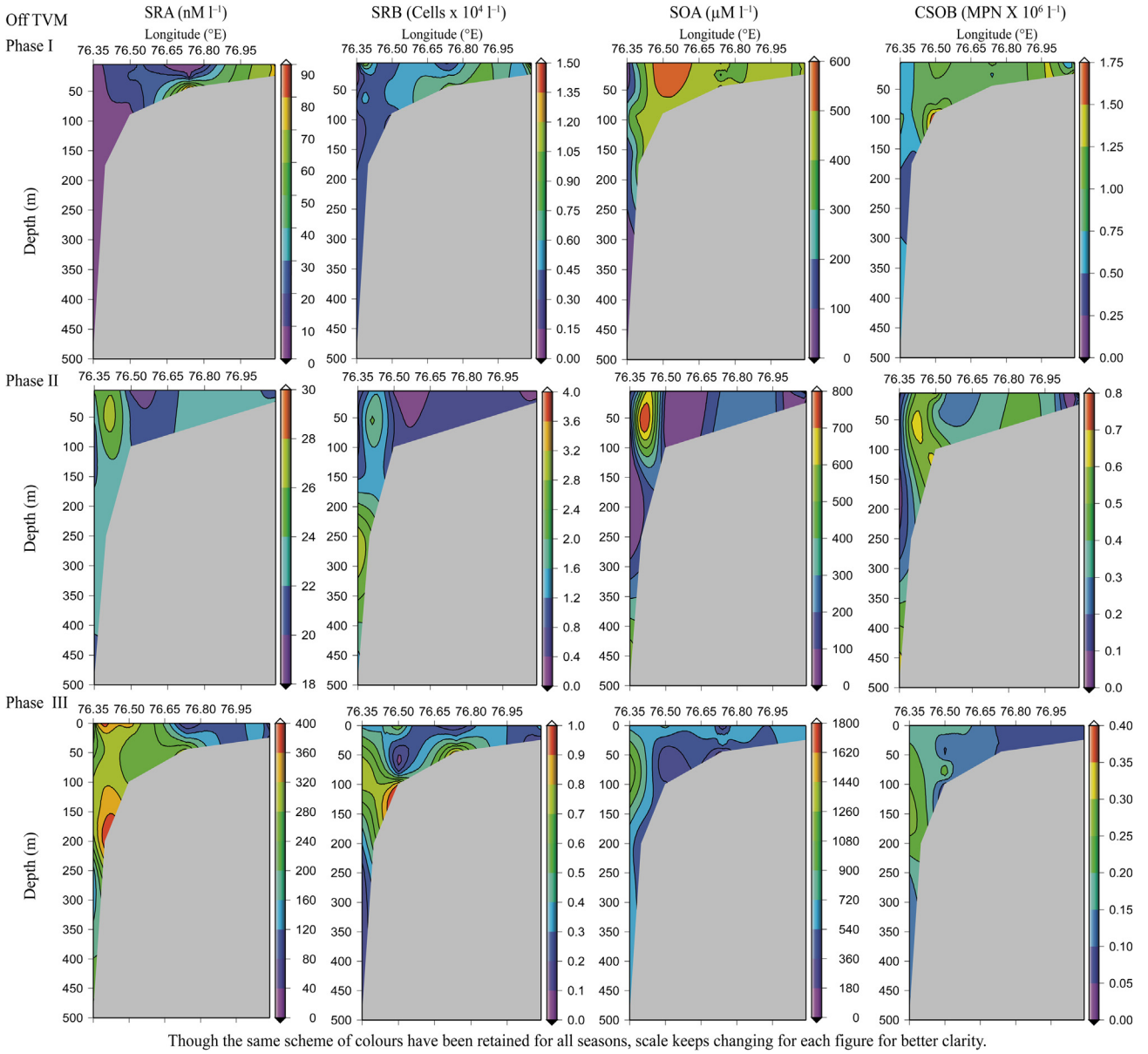

Figure 3 (*Continued*).

phase III suggest that the three phases follow the general paradigm, where the phytoplankton and zooplankton increase in succession. The phytoplankton then get intensely grazed and degraded.

Although TVM is known to be more productive (Gerson et al., 2014), the Chl *a* concentration was generally lower than that off Kochi due to higher grazing activity by mesozooplankton. Consequently, the phaeopigment concentration off TVM was highest (2.98 $\mu g\ l^{-1}$) during phase III and was distributed throughout the water column. However, the water column phaeopigment/Chl *a* ratio, suggest that the degradation processes were more active in phase II.

4.1. Microbiological parameters — abundance

The abundance of TC and TVC are in the range reported earlier by Ducklow et al. (2001). The abundance of SRB ranged from 10^2 to $10^4\ l^{-1}$. Of late, they have been encountered in the aerobic regions hosting anaerobic microniches (Colin et al., 2017; Jørgensen and Parkes, 2010; LokaBharathi and Chandramohan, 1990; Shanks and Reeder, 1993). In the solar lake, the higher abundance of SRB at $10^{6-7}\ l^{-1}$ was reported in the oxic surface waters (Teske et al., 1998). Similarly, Neretin et al. (2007) stated that, the proportion of SRB to total bacteria was 0.1% in the oxic, 0.8–1.9% in the suboxic and 1.2–4.7% in the anoxic zone, where SRB population ranged from $5 \times 10^5\ l^{-1}$–$6.3 \times 10^5\ l^{-1}$ in sub-oxic to anoxic water column of Black Sea. The peak MPN SRB of 2.5×10^9 cells l^{-1} was estimated in the water column of the alpine meromictic Gek-Gel Lake at 33 m depth (Karnachuk et al., 2006). In the present study, the higher abundance of SRB was in the sub-surface waters and accounted for 0.01% of total bacterioplankton. Colin et al. (2017) also stated that SRB thriving in the anoxic sediments may be re-suspended in the oxic water column and transported through oxic

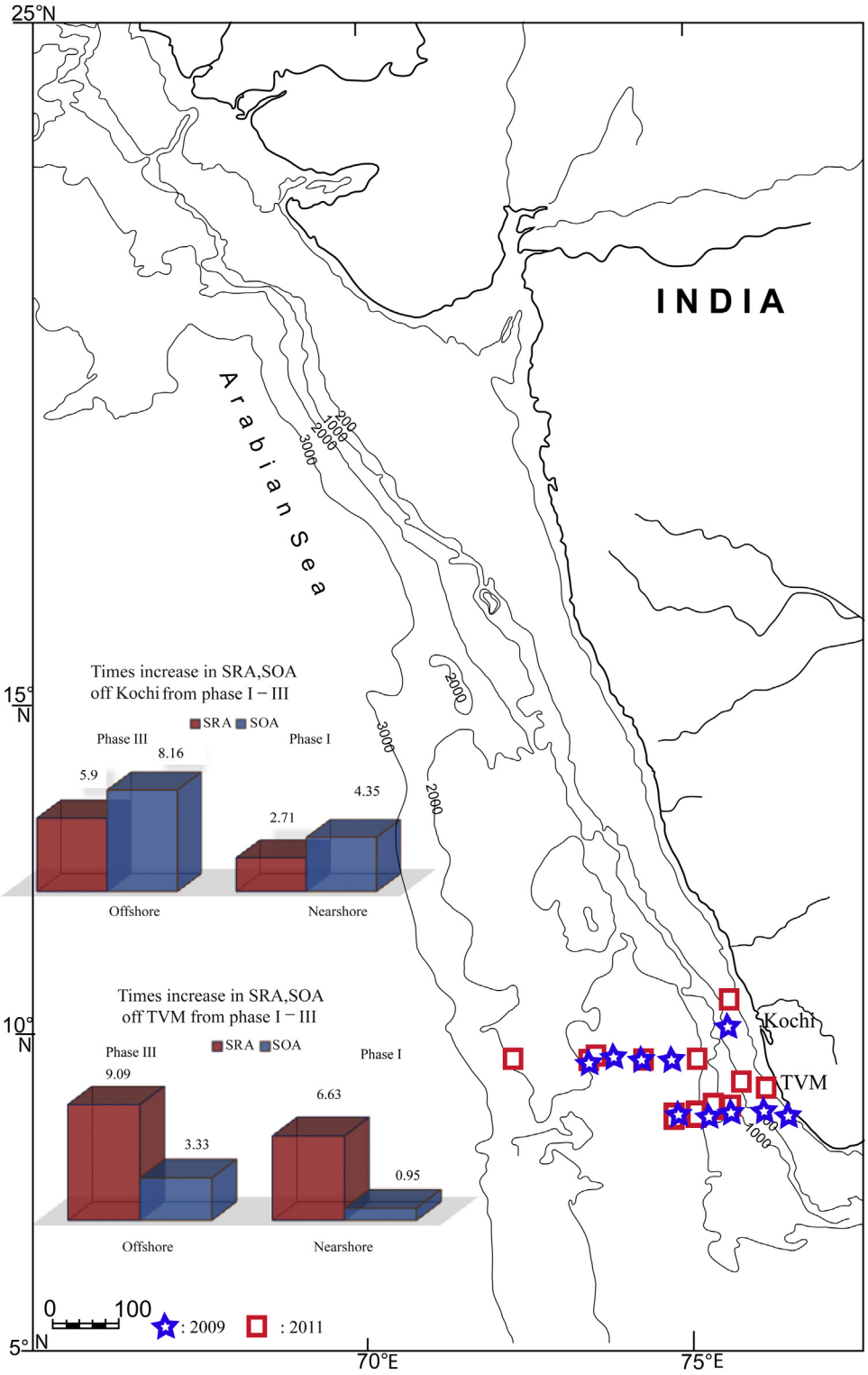

Figure 4 Times increase in sulfate-reducing activity (SRA) and sulfur-oxidizing activity (SOA) from phase I (2009) to phase III (2011) of upwelling at nearshore and offshore of Kochi and Trivandrum (TVM).

marine-influenced waters. Intense interactions among physical, chemical, and biological processes forming strong gradients of the waters may help re-suspend anaerobes to survive in the water column and even maintain their metabolism within anoxic micro-niches. More importantly, SRB can be found in water column either because they have the enzyme superoxide dismutase (Wu et al., 2017) or they have anti-oxidative defense systems (Brioukhanov et al., 2010) or they have the ability to respire oxygen (Cypionka, 2000). Dolla et al. (2006) also stated that SRB can develop molecular strategies to remove oxygen or they use oxygen temporarily for respiration to reduce toxicity.

Table 4 Analysis of variance of sulfate-reducing activity (SRA) and sulfur-oxidizing activity (SOA) during the three phases of upwelling off Kochi and Trivandrum (TVM).

Station	Parameters	Source of variation	SS	df	MS	*F*	*P*-value	*F* crit
Kochi	SRA: Phase I to Phase III	Between groups	13,487.59	1	13,487.59	5.37	**0.0286**	4.22
		Within groups	65,302.40	26	2511.63			
	SOA: Phase I to Phase III	Between groups	6,484,745.00	1	6,484,745.00	66.23	**0.0000**	4.22
		Within groups	2,545,819.00	26	97,916.12			
TVM	SRA: Phase I to Phase III	Between groups	409,652.70	1	409,652.70	39.52	**0.0000**	4.07
		Within groups	435,257.90	42	10,363.28			
	SOA: Phase I to Phase III	Between groups	488,047.70	1	488,047.70	4.55	**0.0387**	4.07
		Within groups	4,501,256.00	42	107,172.80			

While SRB produces sulfide, CSOB can oxidize this toxic end product to polythionates/thiosulfate and sulfate, both aerobically in the presence of oxygen or anaerobically in presence of nitrate (Nelson et al., 2004). Off Kochi and TVM, CSOB did not show any logarithmic variation and ranged from 0.1×10^6 l^{-1} to 0.72×10^6 l^{-1} and 0.15×10^6 l^{-1} to 0.83×10^6 l^{-1} respectively (Fig. 3a and b, Table 2). The free-living marine sulfur oxidizers are abundant in diverse seawater samples including in dark ocean (Swan et al., 2011) and they accounted for 45% of the 16S rRNA gene clones recovered from an OMZ in the South Atlantic and 37% from an anoxic fjord in British Columbia (Lavik et al., 2009; Walsh et al., 2009). They are especially abundant in coastal seawater. Some of the thiosulfate oxidizers oxidize thiosulfate as an auxiliary electron donor to tetrathionate. This allows them to use a greater portion of available organic carbon for biosynthesis rather than for respiration (Jannasch et al., 1991). However, knowledge regarding their abundance, distribution, and ecological role is scarce. Lavik et al. (2009) also identified groups of sulfur oxidizers in the upwelling coastal waters of Namibian shelf and reported chemoautotrophic bacteria to account for ~20% of the bacterioplankton in the sulfidic waters. Their abundance in the non-sulfidic and sulfidic bottom waters was in the range of 10^6 cells l^{-1} to 10^7 cells l^{-1}. Other authors (Arning et al., 2008; Goldhammer et al., 2010; Sievert et al., 2007) noticed the presence of chemolithotrophic sulfur-oxidizing bacteria in the upwelling areas. The present study shows that the culturable sulfur oxidizers are widely present in upwelling waters, accounting for 1% of the bacterio-plankton.

A culturable approach was used for the analysis because the high organic loading in the coastal waters permits relatively high recovery (De Souza et al., 2007). Despite the inherent caveats, the culturable approach retrieves the dominant culturable bacterio-plankton communities as coastal waters have considerable dissolved organic carbon levels (Simu et al., 2005). Besides, new isolates could add to the existing bacterial database which is an important requirement.

4.2. Microbiological parameters — activity

Off Kochi, the SRA values increased over the phases. Off TVM, SRA increased from phase I to phase III with the dip in phase II (Table 2). This decrease was associated with high Chl *a* and phaeopigment concentrations. The SRA off Kochi and TVM are comparable to 13 nM d^{-1} reported by Jørgensen et al. (1991), and 3.5 nM d^{-1} by Albert et al. (1995) in the Black Sea water column and 12 nM d^{-1} in the OMZ waters of Chilean coast by Canfield et al. (2010). However, much higher values of 1569 nM d^{-1} have been reported by Pimenov and Neretin (2006) in these waters.

SOA values were 194, 360, 1151 μM d^{-1} off Kochi and 339, 215 and 560 μM d^{-1} off TVM for the three phases respectively suggesting more dominant oxidation processes off Kochi. The sulfur-oxidizing activity was comparable to the north Atlantic water where 358 μM d^{-1} was reported by Tuttle and Jannasch (1976). High values could be due to the contribution from organic loading from inshore waters that promote both SRA and SOA. Thomas et al. (2013) stated that coastal waters off Kochi receive inshore waters and organic load from backwaters along with more fresh water and nutrients. Perhaps higher inputs of electron donors could account for higher SOA off Kochi, especially, in the presence of nitrate (LokaBharathi et al., 1997).

4.3. Inter-relationship of parameters driving SRA/SRB and SOA/CSOB

4.3.1. Depth

The SRA off Kochi is not depth dependent, while off TVM, SRA is negatively influenced by depth in phase I ($r = -0.315$; $r^2 \times 100 = 10\%$) and phase III (13%). These observations suggest that the signature of upwelling had just begun in phase I and it had dissipated by phase III. However, the SRB off Kochi and TVM are depth dependent in phase I and phase III. These observations suggest that the activity gets relatively more influenced by upwelling than the bacterial abundance. At these stages, the availability of organic matter (PON: $r = 0.820$, $p < 0.001$ and POC: $r = 0.633$, $p < 0.01$ unpublished data by Sam Kamaleson et al., under review) released during phytoplankton growth and degradation at the surface waters facilitates the distribution and intensity of SRA than the abundance of SRB. Moreover, Aluwihare et al. (1997) stated that DOM available in the ocean's surface was at least twice that in the deep sea. Besides, a high abundance of SRB has been noticed earlier by other researchers at the surface in non-upwelling regions (Cypionka, 2000; LokaBharathi and Chandramohan, 1990; Teske et al., 1998).

4.3.2. Temperature

The temperature, on the other hand, influenced SRB distribution positively, being responsible for 36% ($p < 0.05$) of the variation in phase I off Kochi. SRA and SOA are also positively influenced by temperature ($p < 0.05$) in phase I off TVM accounting for 16% and 27% of their variation respectively. Sokolova (2010) suggested that the optimum temperature for SRB was in the range of 27°C–35°C. They also found that the SRA was 6× higher at mesophilic condition than at psychrophilic condition.

4.3.3. Nutrients

Off TVM, nutrients affect the distribution of CSOB in phase I and in phase III and, SRB in phase II. Relationship of ammonium with CSOB evolves from negative to positive from phase I to phase III. The pigment degradation to form phaeopigments increased from 0.23 μM in phase I to 0.57 μM in phase III and is accompanied by more ammonium production in phase III. Perhaps CSOB increases due to the higher rate of nitrification that could provide nitrate as an electron acceptor (Sorokin et al., 2006). The SOA negatively influences ambient phosphate, silicate and nitrate concentrations in phase I (Table 3). In phase II, CSOB positively correlated with nitrite suggesting that the community is capable of nitrate reduction. Krishnan et al. (2008) have shown that intrinsic nitrification can feed denitrification in the coastal waters. Such release has also been noticed by Schutte et al. (2018) along with nitrate accumulation.

Off TVM, both the groups of bacteria act differently with phosphate. In phase I, SOA related negatively with phosphate, while in phase II, SRB related positively (Table 3). Anaerobic oxidation of phosphite to phosphate during sulfate reduction increases soluble phosphate (Schink and Friedrich, 2000). The SRB produce sulfide through SRA which binds with ambient iron thus releasing the iron-bound phosphate (Howarth et al., 2011). However, phosphate could be taken up by CSOB through SOA directly for later use (Glaubitz et al., 2013).

Off TVM, the significant correlation of silicate with SRB and negative correlation with SOA suggests its oxidation to the insoluble state. SRA increases pH during the process and silica solubility also increases with alkalinity (Meier et al., 2012).

Sulfur oxidizers are also known to store phosphate intracellularly and use phosphate for sulfide/thiosulfate oxidation (Goldhammer et al., 2010). In this study also, SRB correlated with phosphate and silicate, indicating its role in releasing these nutrients, which CSOB utilize, thus confirming the synergistic relationship between these two groups.

4.3.4. Chl *a* and phaeopigments

Off Kochi, high Chl *a* values are matched by low concentrations of silicate and nitrate ($r = -0.709$, $p < 0.001$ and $r = -0.521$, $p < 0.05$) suggesting the uptake of these nutrients for chlorophyll build-up and primary productivity. Moreover, iron limited diatom communities that multiply generally deplete silicate long before nitrate (Capone and Hutchins, 2013). The limitation of bio-available iron could be attributed to the increase in SRA over the three phases. Sulfide the product of sulfate reduction is known to readily bind with iron.

The relationships of SRA/SRB with Chl *a*/phaeopigments could be incidental and attributed to the effects of upwelling which could have facilitated the interactions between SRB and the surface-produced chlorophyll. In phase II, the relation between SRB and Chl *a*, phaeopigment was negative due to limited availability or due to faster uptake of break down products of the pigments. The influence of Chl *a* on SRA, changes from negative to positive from phase II to phase III. The observation suggests that when upwelling peaks, it increases the production of pigments and DOC which is turn stimulates SRA at the surface. It should be also noted that SRA and SRB are not deterred by the presence of oxygen (Cypionka, 2000; Sass et al., 2002) especially under DOC/POC replete conditions in upwelling waters.

Both SRA and SRB relate to Chl *a* off TVM due to upwelling linked productivity. Further, anaerobic niches in TSM could support these bacteria and their activity better. Such an observation was however restricted to TVM. Further, phaeopigment which covaries with Chl *a* could also contribute to this association. Up to 43% of the variation in SRA was due to phaeopigment off TVM. Phaeopigments form a part of TSM as evidenced by their inter-relationships. Besides, phaeopigment is a degradative product of Chl *a*, and is a rich source of secondary metabolites for SRB and its activity. Hence both TSM ($r = 0.624$, $p < 0.001$) and phaeopigments ($r = 0.652$, $p < 0.001$) influence SRA positively.

On the contrary, the influence of these parameters is negative to CSOB and its activity as these forms are less heterotrophic and more autotrophic to mixotrophic (Nelson et al., 2004). Incidentally, phase I off Kochi was an exception. Thus, on the whole, chlorophyll *a* and phaeopigments have a positive influence on SRB (32%) and SRA (43%) respectively while, nutrients like nitrate, phosphate and silicate responded and interacted with CSOB and SOA.

4.4. Microbial interaction — abundance

The interaction of TC with environmental parameters was obvious off TVM in the initial phase of upwelling, disappeared in the subsequent phases suggesting disruption of connectivity over the phases. These interactions of TC show that they decrease with depth, and covary with phosphate, nitrate and phaeo/Chl *a* ratio. However, silicate and ammonium interacted positively being responsible for 36% variation of TC. Intriguingly, such interactions were not observed in all phases off Kochi perhaps attributed to higher riverine forcing than upwelling.

4.5. Reducing activity

In coastal areas, the reducing activity due to hypoxia (oxygen concentration $\leq$ 2.8 ml l^{-1} or 63 μM) has been increasing (Banse et al., 2014; Middelburg and Levin, 2009). Naqvi et al. (2010) also stated that the coastal hypoxia has been developing in the most productive part of the Indian Ocean (Arabian Sea). Moreover, recent studies on the Gulf of Arabian Sea proved that the dead zone (OMZ) has expanded more than expected, raising a serious threat on local fisheries and ecosystems (Queste et al., 2018). As low as 10 μM of oxygen (0.23 ml l^{-1}) was detected in phase II and 15 μM (0.33 ml l^{-1}) in phase III off TVM. Understanding how the sulfur cycle

operates in such an environment is important to predict how these expanding areas will impact organic matter degradation. It should be noted that the borders of the OMZ in the Arabian Sea, south-west coast of India have extended from 18°N to 11°N as on 2004 (Banse et al., 2017) and have not yet been known to impinge the waters off Kochi (10°N) and Trivandrum (08°N). However, to distinguish low oxygen zones from true anoxic zones, which are rich in nitrite, the latter are often referred to as anoxic marine zones (AMZs) by Ulloa et al. (2012). OMZ waters are characterized by $\leq$63 μM of oxygen and $\leq$0.02 μM of nitrite (Banse et al., 2014) and AMZs are characterized by $\leq$2 μM oxygen and $\geq$0.5 μM of nitrite (Ulloa et al., 2012). In the current study, the average oxygen concentration off TVM was 1.48 ± 1.55 ml l^{-1} and 1.93 ± 1.86 ml l^{-1} in phase II and III respectively. The corresponding minimum oxygen concentrations were 10 μM and 15 μM respectively. The average nitrite concentrations were 1.69 ± 0.67 μM and 0.34 ± 0.42 μM in phase II and III respectively. The minimum nitrite concentration was 1.24 μM in phase II and it was non-detectable in phase III. Hence, it is suggested that the coastal waters examined in this study could probably have a status between OMZ and AMZ in patches intermittently. Noticeably, some pockets of hypoxic conditions defined by oxygen concentration ~45 μM have also been encountered off Kochi. However, physicochemical and microbiological processes perhaps prevent the spread of hypoxic pockets over time and space.

The spread of reducing activity off Kochi could be mostly due to the riverine influence and anthropogenic effects (Thomas et al., 2013), while off TVM could be more a consequence of upwelling. Here, the SRA gets mitigated both by stronger physical forcing that carries the water offshore and by SOA which counters the effect of SRA partially.

Thus, the coastal waters off TVM and Kochi are influenced by sulfate-reducing and sulfur-oxidizing activities. Prevalence of both SRB and CSOB has also been documented in the Chilean coast by Canfield et al. (2010) and Aldunate et al. (2018). Though they state that the sulfur cycle was cryptic with no obvious *in situ* chemical expression, the metagenomic results suggest an active sulfur cycle. Perhaps SOA counters SRA so effectively with a quick turn over of sulfide that the sulfur cycle appeared cryptic in those waters (Crowe et al., 2018).

4.6. Relation between SRA and SOA

Sulfur oxidation is one of the important processes which occur in most productive upwelling areas (Arning et al., 2008). Generally, SRA and SOA increase over the three phases of observation. Wherever the sulfate reduction is prominent, it is accompanied by sulfur-oxidizing activity (Alsenz et al., 2015; Arning et al., 2008). Ulloa et al. (2012) also identified sulfide (dsr), thiosulfate oxidation (sox) and sulfate-reducing (aprA) genes by metagenomics of Arabian Sea waters. Similarly, in the present study SRA and SOA have been shown to occur simultaneously except that, the increase in rates are different in different phases and transects. The increase ~7× in SRA and 1.7× in SOA, return the redox of the ecosystem only partially off TVM. Off Kochi, ~6× increase in SOA is more than the ~4× increase in SRA thus mitigating an increased effect of SRA (Fig. 4). Further, ANOVA of whole column SRA and SOA emphasizes that the increase in SRA is more statistically significant ($p < 0.001$) off TVM than off Kochi ($p < 0.02$) and the increase in SOA is more significant off Kochi ($p < 0.001$) than off TVM ($p < 0.03$). Thus, the present study highlights the importance of SOA. Besides, it is imperative that sulfide/thiosulfate-oxidation proceeds at much faster rates than sulfate-reduction, as the end product sulfide could be detrimental to biotic variables even at low concentrations. Our studies show that bacterial communities responsible for sulfur-cycle, shift over the course of the upwelling season from a sulfate-reduction dominated regime to a more sulfur-oxidation-dominated system in the later stage.

5. Conclusion

The self-organization of a system runs through several layers mainly, primary, secondary and tertiary levels, which have been well documented. However, the layer below the primary level that is the microbial layer which is responsible for the important processes of mineralization and rejuvenation is hardly chronicled. This is a first attempt to elucidate the contribution of bacteria of the sulfur cycle at the activity level in these waters. Thus we have been able to detect a general temporal increase in oxidizing activity over three different phases accompanied by general spatial spread towards offshore. However, due to eutrophication, these couplings could weaken, and there is a tendency for the increase and spread of reducing activity. Future observations beyond upwelling seasons including transects south of 10°N could further enlighten our inference. Our studies reiterate the ecological rebound and recovery that happens after upwelling due to bacterial processes.

Acknowledgements

Authors express their gratitude to the Director of CSIR-NIO, for the encouragement and the facilities provided. The financial support by the Ministry of Earth Sciences is acknowledged. This work was carried out under the Project number GAP 2033 funded by CMLRE (MoES), and 'Supra Institutional project' SIP 1301 of Council of Scientific and Industrial Research (CSIR)-NIO, Goa, India. CMLRE, Kochi, is acknowledged for the cooperation extended by the participants and crew of FORV Sagar Sampada (#267, #276, #282). Dr. Gauns MU is kindly acknowledged for Chl *a* values. The contributions of anonymous reviewers and editor of *Oceanologia* have considerably improved the manuscript. Sam Kamaleson acknowledges CSIR for Senior Research Fellowship. This is NIO contribution No. 6368.

References

Albert, D.B., Taylor, C., Martens, C.S., 1995. Sulfate reduction rates and low molecular weight fatty acid concentrations in the water column and surficial sediments of the Black Sea. Deep Sea Res. Pt. I 42 (7), 1239–1260, http://dx.doi.org/10.1016/0967-0637(95)00042-5.

Aldunate, M., De la Iglesia, R., Bertagnolli, A.D., Ulloa, O., 2018. Oxygen modulates bacterial community composition in the

coastal upwelling waters off central Chile. Deep Sea Res. Pt. II 156, 68—79, http://dx.doi.org/10.1016/j.dsr2.2018.02.001.

Alsenz, H., Illner, P., Ashckenazi-Polivoda, S., Meilijson, A., Abramovich, S., Feinstein, S., Almogi-Labin, A., Berner, Z., Püttmann, W., 2015. Geochemical evidence for the link between sulfate reduction, sulfide oxidation and phosphate accumulation in a Late Cretaceous upwelling system. Geochem. Trans. 16, 1—13, http://dx.doi.org/10.1186/s12932-015-0017-1.

Aluwihare, L.I., Repeta, D.J., Chen, R.F., 1997. A major biopolymeric component to dissolved organic carbon in surface sea water. Nature 387, 166—169, http://dx.doi.org/10.1038/387166a0.

Arning, E.T., Birgel, D., Schulz-Vogt, H.N., Holmkvist, L., Jørgensen, B.B., Larson, A., Peckmann, J., 2008. Lipid biomarker patterns of phosphogenic sediments from upwelling regions. Geomicrobiol. J. 25, 69—82, http://dx.doi.org/10.1080/01490450801934854.

Banse, K., Naqvi, S.W.A., Narvekar, P.V., Postel, J.R., Jayakumar, D. A., 2014. Oxygen minimum zone of the open Arabian Sea: variability of oxygen and nitrite from daily to decadal time scales. Biogeosci. Discuss. 11 (8), 2237, http://dx.doi.org/10.5194/bg-11-2237-2014.

Banse, K., Naqvi, S.W.A., Postel, J.R., 2017. A zona incognita surrounds the secondary nitrite maximum in open-ocean oxygen minimum zones. Deep Sea Res. Pt. I 127, 111—113, http://dx.doi.org/10.1016/j.dsr.2017.07.004.

Bottrell, S.H., Smart, P.L., Whitaker, F., Raiswell, R., 1991. Geochemistry and isotope systematics of sulfur in the mixing zone of Bahamian blue holes. Appl. Geochem. 6 (1), 97—103, http://dx.doi.org/10.1016/0883-2927(91)90066-x.

Brioukhanov, A.L., Pieulle, L., Dolla, A., 2010. Antioxidative defense systems of anaerobic sulfate reducing microorganisms. In: Mendez Vilas, A. (Ed.), Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Microbiology Book Series, volume 1. Formatex Research Center, Badajoz, Spain, 148—159.

Canfield, D.E., 2001. Isotope fractionation by natural populations of sulfate-reducing bacteria. Geochim. Cosmochim. Acta 65 (7), 1117—1124, http://dx.doi.org/10.1016/s0016-7037(00)00584-6.

Canfield, D.E., Kristensen, E., Thamdrup, B., 2005. Aquatic geomicrobiology. Adv. Mar. Biol. 48, 1—599, http://dx.doi.org/10.1016/s0065-2881(05)48015-3.

Canfield, D.E., Stewart, F.J., Thamdrup, B., Brabandere, L.D., Dalsgaard, T., Delong, E.F., Revsbech, N.P., Ulloa, O., 2010. A cryptic sulfur cycle in oxygen-minimum zone waters off the Chilean Coast. Science 330, 1375—1378, http://dx.doi.org/10.1126/science.1196889.

Capone, D.G., Hutchins, D.A., 2013. Microbial biogeochemistry of coastal upwelling regimes in a changing ocean. Nat. Geosci. 6, 711—717, http://dx.doi.org/10.1038/ngeo1916.

Colin, Y., Goñi-Urriza, M., Gassie, C., Carlier, E., Monperrus, M., Guyoneaud, R., 2017. Distribution of sulfate-reducing communities from estuarine to Marine Bay Waters. Microb. Ecol. 73, 39—49, http://dx.doi.org/10.1007/s00248-016-0842-5.

Crowe, S.A., Cox, R.P., Jones, C., Fowle, D.A., Santibañez-Bustos, J. F., Ulloa, O., Canfield, D.E., 2018. Decrypting the sulfur cycle in oceanic oxygen minimum zones. ISME J. 12, 2322—2329, http://dx.doi.org/10.1038/s41396-018-0149-2.

Cypionka, H., 2000. Oxygen respiration by desulfovibrio species. Annu. Rev. Microbiol. 54, 827—848, http://dx.doi.org/10.1146/annurev.micro.54.1.827.

De Souza, M.J., LokaBharathi, P.A., Shanta, N., Chandramohan, D., 2007. "Trade-off" in Antarctic bacteria: limnetic psychrotrophs concede multiple enzyme expressions for multiple metal resistance. Biometals 20 (6), 821—828, http://dx.doi.org/10.1007/s10534-006-9045-8.

Dolla, A., Fournier, M., Dermoun, Z., 2006. Oxygen defense in sulfate reducing bacteria. J. Biotechnol. 126, 87—100, http://dx.doi.org/10.1016/j.jbiotec.2006.03.041.

Ducklow, H.W., Smith, D.C., Campbell, L., Landry, M.R., Quinby, H. L., Steward, G.F., Azam, F., 2001. Heterotrophic bacterioplankton in the Arabian Sea: basinwide response to year-round high primary productivity. Deep Sea Res. Pt. II 48 (6—7), 1303—1323, http://dx.doi.org/10.1016/s0967-0645(00)00140-5.

Fortin, D., Rioux, J.P., Roy, M., 2002. Geochemistry of iron and sulfur in the zone of microbial sulfate reduction in mine tailings. Water Air Soil Poll. 2 (3), 37—56, http://dx.doi.org/10.1023/a:1019983024680.

Fossing, H., Gallardo, V.A., Jørgensen, B.B., Hüttel, M., Nielsen, L.P., Schulz, H., Canfield, D.E., Forster, S., Glud, R.N., Gundersen, J. K., Küver, J., Ramsing, N.B., Teske, A., Thamdrup, B., Ulloa, O., 1995. Concentration and transport of nitrate by the mat-forming sulphur bacterium *Thioploca*. Nature 374, 713—715, http://dx.doi.org/10.1038/374713a0.

Gerson, V.J., Madhu, N., Jyothibabu, R., Balachandran, K., Nair, M., Revichandran, C., 2014. Oscillating environmental responses of the eastern Arabian Sea. Indian J. Geo-Mar. Sci. 43, 67—75.

Gibson, G.R., 1990. Physiology and ecology of the sulphate-reducing bacteria. J. Appl. Microbiol. 69, 769—797, http://dx.doi.org/10.1111/j.1365-2672.1990.tb01575.x.

Glaubitz, S., Kießlich, K., Meeske, C., Labrenz, M., Jürgens, K., 2013. SUP05 dominates the gamma proteobacterial sulfur oxidizer assemblages in pelagic redoxclines of the Central Baltic and Black Seas. J. Appl. Microbiol. 79 (8), 2767—2776, http://dx.doi.org/10.1111/j.1365-2672.1990.tb01575.x.

Goes, J.I., Thoppil, P.G., Gomes, H., Fasullo, J.T., 2005. Warming of the Eurasian landmass is making the Arabian Sea more productive. Science 308 (5721), 545—547, http://dx.doi.org/10.1126/science.1106610.

Goldhammer, T., Brüchert, V., Ferdelman, T.G., Zabel, M., 2010. Microbial sequestration of phosphorus in anoxic upwelling sediments. Nat. Geosci. 3 (8), 557—561, http://dx.doi.org/10.1038/ngeo913.

Gonsalves, M.J., Paropkari, A.L., Fernandes, C.E.G., LokaBharathi, P. A., Krishnakumari, L., Fernando, V., Nampoothiri, G.E., 2011. Predominance of anaerobic bacterial community over aerobic community contribute to intensify 'oxygen minimum zone' in the eastern Arabian Sea. Cont. Shelf Res. 31, 1224—1235, http://dx.doi.org/10.1016/j.csr.2011.04.011.

Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. Methods of Seawater Analysis. Verlag Chemie, Weinheim, 125 pp.

Hansen, N.W., 1973. Official Standardized and Recommended Methods of Analysis. The Society of Analytical Chemistry, London, 897 pp.

Hobbie, J.E., Daley, R.J., Jasper, S., 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33, 1225—1226.

Howarth, R., Chan, F., Conley, D.J., Garnier, J., Doney, S.C., Marino, R., Billen, G., 2011. Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. Front. Ecol. Environ. 9 (1), 18—26, http://dx.doi.org/10.1890/100008.

Jannasch, H.W., Wirsen, C.O., Molyneaux, S.J., 1991. Chemoautotrophic sulfur-oxidizing bacteria from the Black Sea. Deep Sea Res. Pt. I 38 (2), S1105—S1120, http://dx.doi.org/10.1016/S0198-0149(10)80026-3.

Jørgensen, B.B., Boetius, A., 2007. Feast and famine-microbial life in the deep-sea bed. Nat. Rev. Microbiol. 5 (10), 770—781, http://dx.doi.org/10.1038/nrmicro1745.

Jørgensen, B.B., Fossing, H., Wirscn, C.O., Jannasch, H.W., 1991. Sulfide oxidation in the anoxic Black Sea chemocline. Deep-Sea Res. 38 (2), S1083—S1103, http://dx.doi.org/10.1016/s0198-0149(10)80025-1.

Jørgensen, B.B., Gallardo, V.A., 1999. *Thioploca* spp.: filamentous sulfur bacteria with nitrate vacuoles. FEMS Microbiol. Ecol. 28 (4), 301—313, http://dx.doi.org/10.1016/s0168-6496(98)00122-6.

Jørgensen, B.B., Parkes, R.J., 2010. Role of sulfate reduction and methane for anaerobic carbon cycling in eutrophic fjord

sediments (Limfjorden, Denmark). Limnol. Oceanogr. 55, 1338–1352, http://dx.doi.org/10.4319/lo.2010.55.3.1338.

Karnachuk, O.V., Pimenov, N.V., Iusupov, S.K., Frank, I., Puhakka, J. A., Ivanov, M.V., 2006. Distribution, diversity, and activity of sulfate-reducing bacteria in the water column in Gek-Gel Lake, Azerbaijan. Microbiology 75 (1), 82–89, http://dx.doi.org/10.1134/s0026261706010152.

King, G.M., 2001. Radiotracer assays (35S) of sulfate reduction rates in marine and freshwater sediments. Method. Microbiol. 30, 489–500, http://dx.doi.org/10.1016/s0580-9517(01)30059-4.

Kogure, K.U., Simidu Taga, N., 1984. An improved direct viable count method for aquatic bacteria. Archives of Hydrobiol 102, 117–122.

Krishnan, K.P., Fernandes, S., LokaBharathi, P.A., Krishna Kumari, L., Shanta, N., Pratihari, A.K., Rao, B.R., 2008. Anoxia over the western continental shelf of India: bacterial indications of intrinsic nitrification feeding denitrification. Marine Environ. Res. 65 (5), 445–455, http://dx.doi.org/10.1016/j.marenvres.2008.02.003.

Kumar, S.P., Ramaiah, N., Gauns, M., Sarma, V.V.S.S., Muraleedharan, P.M., Raghukumar, S., Kumar, M.D., Madhupratap, M., 2001. Physical forcing of biological productivity in the Northern Arabian Sea during the Northeast Monsoon. Deep Sea Res. Pt. II 48 (6), 1115–1126, http://dx.doi.org/10.1016/S0967-0645(00)00133-8.

Lachkar, Z., Lévy, M., Smith, S., 2018. Intensification and deepening of the Arabian Sea Oxygen Minimum Zone in response to increase in Indian monsoon wind intensity. Biogeosciences 15 (1), 159–186, http://dx.doi.org/10.5194/bg-15-159-2018.

Lavik, G., Stuhrmann, T., Brüchert, V., Van der Plas, A., Mohrholz, V., Lam, P., Mussmann, M., Fuchs, B.M., Amann, R., Lass, U., Kuypers, M.M., 2009. Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. Nature 457, 581–586, http://dx.doi.org/10.1038/nature07588.

LokaBharathi, P.A., 1989. The occurrence of denitrifying colourless sulfur-oxidizing bacteria in marine waters and sediments as shown by the agar shake technique. FEMS Microbiol. Ecol. 62, 335–342, http://dx.doi.org/10.1111/j.1574-6968.1989.tb03388.x.

LokaBharathi, P.A., Chandramohan, D., 1990. Sulfate-reducing bacteria from the Arabian Sea – their distribution in relation to thiosulfate-oxidizing and heterotrophic bacteria. Bull. Mar. Sci. 47, 622–630.

LokaBharathi, P.A., Chandramohan, D., Nair, S., 1988. A preliminary study of anaerobic thiosulfate-oxidizing bacteria as denitrifiers in the Arabian Sea. Geomicrobiol. J. 6, 195–207, http://dx.doi.org/10.1080/01490458809377839.

LokaBharathi, P.A., Nair, S., Chandramohan, D., 1997. Anaerobic sulfide-oxidation in marine colorless sulfur-oxidizing bacteria. J. Mar. Biotechnol. 5, 172–177.

Madhupratap, M., Gauns, M., Ramaiah, N., Kumar, S.P., Muraleedharan, P.M., De Sousa, S.N., Sardessai, S., Muraleedharan, U., 2003. Biogeochemistry of the Bay of Bengal: physical, chemical and primary productivity characteristics of the central and western Bay of Bengal during summer monsoon 2001. Deep Sea Res. Pt. II 50 (5), 881–896, http://dx.doi.org/10.1016/s0967-0645(02)00611-2.

Madhupratap, M., Kumar, S.P., Bhattathiri, P.M.A., Kumar, M.D., Raghukumar, S., Nair, K.K.C., Ramaiah, N., 1996. Mechanism of the biological response to winter cooling in the northeastern Arabian Sea. Nature 384, 549–552, http://dx.doi.org/10.1038/384549a0.

Malik, A., Fernandes, C.E.G., Gonsalves, M.J.B.D., Subina, N.S., Mamatha, S.S., Krishna, K., Varik, S., Kumari, R., Gauns, M., Cejoice, R.P., Pandey, S.S., Jineesh, V.K., Kamaleson, A.S., Vijayan, V., Mukherjee, I., Subramanyan, S., Nair, S., Ingole, B., LokaBharathi, P.A., 2015. Interactions between trophic levels in upwelling and non-upwelling regions during summer monsoon. J. Sea Res. 95, 56–69, http://dx.doi.org/10.1016/j.seares.2014.10.012.

Meier, J., Piva, A., Fortin, D., 2012. Enrichment of sulfate-reducing bacteria and resulting mineral formation in media mimicking pore water metal ion concentrations and pH conditions of acidic pit lakes. FEMS Microbiol. Ecol. 79 (1), 69–84, http://dx.doi.org/10.1111/j.1574-6941.2011.01199.x.

Middelburg, J.J., Levin, L.A., 2009. Coastal hypoxia and sediment biogeochemistry. Biogeosciences 6, 1273–1293, http://dx.doi.org/10.5194/bg-6-1273-2009.

Naqvi, S.W.A., 1991. Geographical extent of denitrification in the Arabian Sea in relation to some physical processes. Oceanologica Acta 14 (3), 281–290.https://archimer.ifremer.fr/doc/00101/21257.

Naqvi, S.W.A., Bange, H.W., Farías, L., Monteiro, P.M.S., Scranton, M.I., Zhang, J., 2010. Marine hypoxia/anoxia as a source of CH_4 and N_2O. Biogeosciences 7, 2159–2190, http://dx.doi.org/10.5194/bg-7-2159-2010.

Nelson, D.C., Amend, J.P., Edwards, K.J., Lyons, T.W., 2004. Sulfide oxidation in marine sediments: geochemistry meets microbiology. Sulfur Biogeochem. 379, 63–81, http://dx.doi.org/10.1130/0-8137-2379-5.63.

Neretin, L.N., Abed, R.M., Schippers, A., Schubert, C.J., Kohls, K., Kuypers, M.M., 2007. Inorganic carbon fixation by sulfate-reducing bacteria in the Black Sea water column. Environ. Microbiol. 9 (12), 3019–3024, http://dx.doi.org/10.1111/j.1462-2920.2007.01413.x.

Pimenov, N.V., Neretin, L.N., 2006. Compositions and activities of microbial communities involved in carbon, sulfur, nitrogen and manganese cycling in the oxic/anoxic interface of the Black Sea. In: Neretin, L.N. (Ed.), Past and Present Water Column Anoxia. Springer, Dordrecht, 501–521, http://dx.doi.org/10.1007/1-4020-4297-3_19.

Queste, B.Y., Vic, C., Heywood, K.J., Piontkovski, S.A., 2018. Physical controls on oxygen distribution and denitrification potential in the north west Arabian Sea. Geophys. Res. Lett. 45 (9), 4143–4152, http://dx.doi.org/10.1029/2017GL076666.

Resplandy, L., Lévy, M., Bopp, L., Echevin, V., Pous, S., Sarma, V.V.S.S., Kumar, D., 2012. Controlling factors of the oxygen balance in the Arabian Sea's OMZ. Biogeosciences 9 (12), 5095–5109, http://dx.doi.org/10.5194/bgd-9-5509-2012.

Sass, A.M., Eschemann, A., Kühl, M., Thar, R., Sass, H., Cypionka, H., 2002. Growth and chemosensory behavior of sulfate-reducing bacteria in oxygen-sulfide gradients. Microbiol. Ecol. 40 (1), 47–54, http://dx.doi.org/10.1111/j.1574-6941.2002.tb00935.x.

Schink, B., Friedrich, M., 2000. Bacterial metabolism: phosphite oxidation by sulphate reduction. Nature 406 (6791), 37, http://dx.doi.org/10.1038/35017644.

Schutte, C.A., Wilson, A.M., Evans, T., Moore, W.S., Joye, S.B., 2018. Deep oxygen penetration drives nitrification in intertidal beach sands. Limnol. Oceanogr. 63 (S1), S193–S208, http://dx.doi.org/10.1002/lno.10731.

Shanks, A.L., Reeder, M.L., 1993. Reducing microzones and sulfide production in marine snow. Mar. Ecol. Prog. Ser. 96, 43–47, http://dx.doi.org/10.3354/meps096043.

Shetye, S.R., Gouveia, A.D., Shenoi, S.S.C., Sundar, D., Michael, G. S., Almeida, A.M., Santanam, K., 1990. Hydrography and circulation off the west coast of India during the southwest monsoon 1987. J. Mar. Res. 48, 359–378, http://dx.doi.org/10.1357/002224090784988809.

Shetye, S.S., Mohan, R., Patil, S., Jena, B., Chacko, R., George, J.V., Noronha, S., Singh, N., Priya, L., Sudhakar, M., 2015. Oceanic pCO_2 in the Indian sector of the Southern Ocean during the austral summer–winter transition phase. Deep Sea Res. Pt. II 118, 250–260, http://dx.doi.org/10.1016/j.dsr2.2015.05.017.

Sievert, S.M., Wierings, E.B.A., Wirsen, C.O., Taylor, C.D., 2007. Growth and mechanism of filamentous-sulfur formation by *Candidatus* Arcobacter sulfidicus in opposing oxygen-sulfide gradients. Environ. Microbiol. 9, 271–276, http://dx.doi.org/10.1111/j.1462-2920.2006.01156.x.

Simu, K., Holmfeldt, K., Zweifel, U.L., Hagström, A., 2005. Culturability and coexistence of colony-forming and single-cell marine bacterioplankton. Appl. Environ. Microbiol. 71, 4793–4800, http://dx.doi.org/10.1128/aem.71.8.4793-4800.2005.

Sokolova, E.A., 2010. Influence of temperature on development of sulfate-reducing bacteria in the laboratory and field in winter. Contemp. Probl. Ecol. 3 (6), 631—634, http://dx.doi.org/10.1134/s1995425510060032.

Sorokin, D.Y., Tourova, T.P., Lysenko, A.M., Muyzer, G., 2006. Diversity of culturable halophilic sulfur-oxidizing bacteria in hypersaline habitats. Microbiology 152, 3013—3023, http://dx.doi.org/10.1099/mic.0.29106-0.

Strickland, J.D.H., Parsons, T.R., 1977. A Practical Handbook of Seawater Analysis, 2nd ed. Fisheries Research Board of Canada, Ottawa, 21 pp., http://publications.gc.ca/pub?id=9.581564&sl=0.

Swan, B.K., Martinez-Garcia, M., Preston, C.M., Sczyrba, A., Woyke, T., Lamy, D., Reinthaler, T., Poulton, N.J., Masland, E.D., Gomez, M.L., Sieracki, M.E., DeLong, E.F., Herndl, G.J., Stepanauskas, R., 2011. Potential for chemolithoautotrophy among ubiquitous bacteria lineages in the dark ocean. Science 333 (6047), 1296—1300, http://dx.doi.org/10.1126/science.1203690.

Teske, A., Ramsing, N.B., Habicht, K., Fukui, M., Küver, J., Jørgensen, B.B., Cohen, Y., 1998. Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl. Environ. Microbiol. 64 (8), 2943—2951, PMID: 9687455.

Teske, A., Wawer, C., Muyzer, G., Ramsing, N.B., 1996. Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. Appl. Environ. Microbiol. 62 (4), 1405—1415, PMID: 8919802.

Thomas, L.C., Padmakumar, K.B., Smitha, B.R., Asha Devi, C.R., Bijoy Nandan, S., Sanjeevan, V.N., 2013. Spatio-temporal variation of microphytoplankton in the upwelling system of the southeastern Arabian Sea during the summer monsoon of 2009. Oceanologia 55 (1), 185—204, http://dx.doi.org/10.5697/oc.55-1.185.

Tuttle, J.H., Jannasch, H.W., 1976. Microbial utilization of thiosulfate in the deep sea. Limnol. Oceanogr. 21 (5), 697—701, http://dx.doi.org/10.4319/lo.1976.21.5.0697.

Ulloa, O., Canfield, D.E., DeLong, E.F., Letelier, R.M., Stewart, F.J., 2012. Microbial oceanography of anoxic oxygen minimum zones. PNAS 109 (40), 15996—16003, http://dx.doi.org/10.1073/pnas.1205009109.

Visscher, P.T., Prins, R.A., Gemerden, H., 1992. Rates of sulfate reduction and thiosulfate consumption in a marine microbial mat. FEMS Microbiol. Ecol. 9 (4), 283—294, http://dx.doi.org/10.1111/j.1574-6941.1992.tb01763.x.

Walsh, D.A., Zaikova, E., Howes, C.G., Song, Y.C., Wright, J.J., Tringe, S.G., Tortell, P.D., Hallam, S.J., 2009. Metagenome of a versatile chemolithoautotroph from expanding oceanic dead zones. Science 326, 578—582, http://dx.doi.org/10.1126/science.1175309.

Walsh, J.J., Dieterle, D.A., Müller-Karger, F.E., Bohrer, R., Bissett, W.P., Varela, R.J., Aparicio, R., Díaz, R., Thunell, R., Taylor, G.T., Scranton, M.I., Fanning, K.A., Peltzer, E.T., 1999. Simulation of carbon/nitrogen cycling during spring upwelling in the Cariaco Basin. J. Geophys. Res. 104, 7807—7825, http://dx.doi.org/10.1029/1998jc900120.

Wiggert, J.D., Murtugudde, R.G., McClain, C.R., 2002. Processes controlling interannual variations in wintertime (Northeast Monsoon) primary productivity in the central Arabian Sea. Deep Sea Res. Pt. II: Top. Stud. Oceanogr. 49 (12), 2319—2343, http://dx.doi.org/10.1016/s0967-0645(02)00039-5.

Winch, S., Mills, H.J., Kostka, J.E., Fortin, D., Lean, D.R.S., 2009. Identification of sulfate-reducing bacteria in methylmercury-contaminated mine tailings by analysis of SSU rRNA genes. FEMS Microbiol. Ecol. 68 (1), 94—107, http://dx.doi.org/10.1111/j.1574-6941.2009.00658.x.

Wu, J., Liu, H., Wang, P., Zhang, D., Sun, Y., Li, E., 2017. Oxygen reduction reaction affected by sulfate-reducing bacteria: different roles of bacterial cells and metabolites. Indian J. Microbiol. 57 (3), 344—350, http://dx.doi.org/10.1007/s1208.

Wurl, O., 2009. Practical Guidelines for the Analysis of Seawater. CRC Press, Ottawa, 408 pp.

Yentsch, C.D., Menzel, D.W., 1963. A method for determination of chlorophyll *a*nd phaeophytin by fluorescence. Deep Sea Res. 10, 221—231, http://dx.doi.org/10.1016/0011-7471(63)90358-9.