Light stress induced DMS(P) production in *Skeletonema costatum*: An experimental approach and field observation

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Abstract

Dimethylsulphide is a dominant biogenic sulphur anti-greenhouse gas produced by marine phytoplankton. A non-axenic culture of *Skeletonema costatum* was studied to comprehend the effects of different growth stages and light stress on DMSP/DMS production. The intracellular DMSP concentration increased during late exponential to mid-stationary phase and attained a maximum $(0.59 \text{ pg S cell}^{-1})$ during the stationary phase, indicating more contribution from actively dividing smaller cells. Likewise, exposure to first light after a 12-hour dark phase caused stress, invariably leading to elevated levels of DMS (~9 fold). These observations were upheld by additional laboratory and field experiments, and a field time-series observation, which recorded higher DMS concentrations during exposure to first light after a dark cycle and during early mornings, respectively. While our study depicts the variable DMSP and DMS concentrations during different growth stages of *S. costatum*, it gives new information on the effect of light stress on DMS production.

Keywords: Culture, Diatom, DMS, DMSP, Light stress, Skeletonema costatum

Highlights

- Maximum DMSP production occurred during late exponential to mid-stationary phase
- Actively dividing smaller cells with a higher surface-to-volume ratio produce DMSP
- First light exposure induces DMS production in Skeletonema costatum
- Laboratory experiments and field time series indicate a similar result
- More research is needed to understand the implications on DMS flux and budget

List of abbrevations

- ASTS Arabian Sea time series station
- CaTS Candolim time series station

Chl - Chlorophyll

- DMS Dimethylsulphide
- DMSP Dimethylsulphoniopropionate
- $DMSP_d-Dissolved\ Dimethyl sulphonio propionate$
- $DMSP_p Particulate Dimethyl sulphonio propionate$
- $DMSP_t Total Dimethyl sulphonio propionate$

1. Introduction

Dimethylsulphide (DMS) a dominant biogenic sulphur gas is well known for its role in the radiation balance of the Earth through its involvement in the production of aerosols and clouds (Charlson et al., 1987; Gondwe et al., 2003; Hoffmann et al., 2016). It is produced in the oceanic surface layers from its precursor dimethylsulphoniopropionate (DMSP), which in turn is produced primarily by marine phytoplankton (Zhang et al., 2019). DMSP in phytoplankton is considered to have multifunctional properties such as osmoregulation, cryoprotection, and/or as an antioxidant (Vairavamurthy et al., 1985; Kirst et al., 1991; Sunda et al., 2002, Bullock et al., 2017). Despite the biogenic origin of DMSP and in turn, DMS, both show poor or no correlation with Chl *a* (Kwint and Kramer, 1996; Simo et al., 1997; Townsend and Keller, 1996; Uher et al., 2006; Shenoy et al., 2002; Bepari et al., 2020). Different groups of phytoplankton produce varying concentrations of DMSP. According to Liss et al. (1993), potential DMSP production follows the order: Coccolithophores > Pheocystis > Dinoflagellates > Diatoms. Moreover, Chlorophytes, Rhodophytes, Chrysophytes, and Prasinophytes are also known to produce DMSP (Mallin and Kirst, 1997; Shenoy et al., 2006).

So far, most of the DMS/DMSP studies from the north Indian ocean focused on their spatial and temporal distributions (Shenov et al., 2002; 2006; Shenov and Kumar, 2007, Bepari et al., 2020, Naik et al., 2020) and sparse information is known about factors influencing/affecting DMS production (Stefels et al., 2007; Zhuang et al., 2011). Several environmental factors such as light intensity, salinity, temperature and nutrient availability are known to cause stress to the phytoplankton influencing DMS/DMSP production (Stefel and Van, 1998; Sunda et al., 2002; 2007; Bucciarelli and Sunda, 2003; Stefels et al., 2007; Harada et al., 2009; Zhuang et al., 2011, McParland and Levine, 2019). A study carried out by Karsten et al. (1990; 1991), reported that the intracellular build-up of DMSP in phytoplankton is light-dependent, where high DMSP content was recorded during the maximum photoperiod. Also, a substantial rise in DMSP concentration in phytoplankton was recorded upon re-exposure to light after the prolonged dark period. On the other hand, bacteria can rapidly utilize DMSP and DMS with short turnover periods. While microbial consumption results in the production of DMS, most of the DMSP is metabolised for the carbon and sulphur requirement (Hatton et al., 2012; Ma et al., 2022). Additionally, ultraviolet B (UVB), ultraviolet A (UVA) and photosynthetically active radiation (PAR) are also known to degrade DMS in surface waters, with UVA and PAR being the dominant drivers of DMS degradation (Xu et al., 2021). Most of the DMS is oxidised in seawater with a small fraction of it being emitted to the atmosphere (Ma et al., 2022). While there are studies available on the increase in DMS concentration during summer due to prolonged light availability (Jarnikova et al. 2018), data on the effects of first light exposure (soon after the dark period) on DMS production is still lacking.

The present study from the coastal waters of the central west coast of India was conducted to record the concentration of DMS and DMSP during different growth stages and stress experienced by the experimental organism: Skeletonema costatum. Subrahmanyan (1946) reported the presence of S. costatum in Indian waters (Arabian Sea and Bay of Bengal), contributing ~1% to the total phytoplankton community. The diatom genus Skeletonema Greville emended by Sarno and Zingone is commonly found in coastal habitats throughout the temperate and tropical regions (Hasle, 1973; Naik et al., 2010). It is a cosmopolitan diatom (Tomas, 1997) and can adapt to a wide range of salinity (Yang et al., 2011). While diatoms are generally regarded as low DMSP and DMS producers, they make up for the dominant phytoplankton in the tropical seas and thus contribute significantly to DMS production (Shenoy et al. 2006). Experiments performed to investigate the role of dust aerosol additions, and ocean acidification on phytoplankton have shown to promote diatom growth and increased production of DMSP and DMS in the Western Pacific Ocean (Gao et al., 2021). Although diatoms are not among the recognized group of phytoplankton contributing to DMSP and DMS production (Keller et al., 1989; Yang et al., 2011); past studies demonstrated that diatoms could be the major contributors to the DMSP and DMS pool, as they dominate the phytoplankton flora (Shenoy et al., 2006; Zhang et al., 2008; Yang et al., 2009; 2011). Our research about the influence of light stress on DMSP and DMS production will help us to understand the potential role of S. costatum and light stress in sulphur cycling. Also, it can provide a clue to explain the variation in DMSP/DMS concentration during S. costatum blooms.

The original aim of the experiment was to document the DMSP and DMS production during different growth stages of the *S. costatum* culture. However, during the experiment we observed a sudden peak in DMS concentration when the culture was switching over from dark to light phase. The present paper discusses in detail how the initial light exposure after the dark phase, both in nature as well as in the laboratory, influences DMS production.

2. Materials and methods

2.1 Isolation and culturing Skeletonema costatum

Uni-algal (non-axenic) culture of *Skeletonema costatum* was obtained by cell picking method under an inverted microscope (Olympus IX51) from the Candolim time series (CaTS)-G5 station located 12 km off Goa, India, which experiences seasonal hypoxia/suboxia/anoxia towards the end of southwest monsoon every year (Naqvi *et al.*, 2006). The isolated cells were then cultured in an F/2 medium at 25°C, and 1000 Lux light intensity was then used for the experimental purpose (see below).

2.2 Experimental setup

S. costatum was cultured in a 2000 mL culture flask in triplicate (SF-1, SF-2 and SF-3); 20 mL of inoculum from the exponential growth phase of culture was added to 1000 mL of F/2 media having a salinity of 35 and incubated at 25°C and 1000 Lux light intensity. Refereeing to Pal et al. (2016), the cultured cells were exposed to optimum light intensity (1000 Lux) to facilitate active growth and reproduction. Experimental flasks were subjected to 12 hours of light (9:01 AM - 9:00 PM) and dark cycle (9:01 PM – 9:00 AM). These culture flasks were then sub-sampled for DMS, DMSP (total, dissolved and particulate), cell count, cell bio-volume, and Chlorophyll (Chl) *a* estimation at 9:15 AM (SF-1), 9:45 AM (SF-2) and 10:15 AM (SF-3). To confirm the light stress signal during the culture run, the sub-sampling times of the first two flasks (SF-1 and SF-2) were switched, thus the third flask (SF-3) can be used as the control.

2.3 DMS and DMSP analysis

DMS and DMSP samples were collected in amber-coloured glass bottles. Care was taken during sample collection to minimize any kind of atmospheric contamination. Samples were filtered through 2 μ m filter paper; the filtrate and filter paper with residue were analyzed for dissolved (DMSP_d) and particulate (DMSP_p) fractions, respectively. The analyses were carried out by using Gas Chromatography (Shimadzu GC 2010) fitted with a flame photometric detector (FPD). DMS and DMSP were analyzed according to the method given by Turner et al. (1990) and Shenoy et al. (2002). Nitrogen gas was used to purge a measured volume of the culture sub-sample. The stripped sulphur gases were dried using three different moisture traps and separated on a Chromosil 330 column and detected by a flame photometric detector. Following DMS measurement, DMSP was hydrolyzed to DMS by adding 1 mL of 10 M NaOH to the same sample with immediate purging for 20 min. This results in the cleavage of DMSP into DMS and acrylic acid. Tests revealed close to 100 % conversion to DMS from DMSP.

2.4 Cell count, bio-volume measurement and Chl a estimation

Samples for cell count and bio-volume measurement were preserved immediately with acid Lugol's Iodine (1%) and were examined using the Sedgewick rafter counting chamber under an inverted microscope (Olympus IX 51, 200X). Bio-volume was measured using NIKON inverted microscope with NIS camera software. Chl *a* concentrations were determined fluorometrically (Turner Designs,

USA) by filtering 5 mL of the sample through GF/F filter paper. The extraction of pigments was carried out for 24 h in 90% acetone at -20°C in the dark (JGOFS, 1994).

2.5 Impact of light on laboratory cultures and environmental samples

In addition to the culture experiment mentioned above (2.2), environmental samples (coastal/open ocean) and uni-algal cultures of diatoms and dinoflagellates kept in the laboratory were sampled during the dark and early light periods for determining DMS concentrations.

2.6 Time series experiment at Arabian Sea time-series station

An environmental time-series study was carried out at the Arabian time-series station (ASTS; 17°N 68°E) in the central Arabian Sea, where water samples were collected every 2 hours for 2 days and analysed for DMS, DMSP_t, and Chl *a* concentration. Data for solar insolation was taken from the AWS system installed onboard RV Sindhu Sadhana.

3. Results and discussion:

3.1 Skeletonema costatum growth pattern

The S-shaped growth curve observed during the experimental period (20 days) showed variation in Chl *a* and cell abundance during the 4 growth stages: lag phase (days 1-3), exponential growth phase (days 4-7), stationary growth phase (days 8-16) and senescent phase (days 17-18, Fig. 1). Maximum Chl *a* concentration ($148 \pm 29 \ \mu g \ L^{-1}$) was observed during the late stationary growth phase (day 16). The cell abundance of *S. costatum* varied between 170 and 2855 Cells x 10⁵ L⁻¹ with maximum cell abundance being observed during the stationary phase of the culture run. The cell bio-volume varied from 31 to 48 μm^3 , recording a maximum on day 5 (mid-exponential growth phase), suggesting active growth and reproduction of cells.







Fig. 1b. Temporal variation bio-volume in *Skeletonema costatum* during the light stress experiment

3.2 Variation of DMSP (total, particulate and dissolved) at different growth phases

The concentration of $DMSP_t$, $DMSP_p$, and $DMSP_d$ in *S. costatum* culture ranged from 89.2 – 2498.9 nM, 8.4 – 1195.0 nM, and 5.0 – 40.4 nM, respectively (Fig. 2). A steady increase in $DMSP_t$ concentration from 89.2 to 2498.9 nM was recorded from lag to senescent phase with a steep increase observed between days 3 and 10. Low $DMSP_d$ was recorded during the initial stages, which increased during the stationary and senescent phase of the culture. The high $DMSP_d$ concentration (40.4 nM) during the senescent phase can be attributed to the ageing and lysis of cells releasing intracellular DMSP into the culture medium (Zhuang et a., 2011). Similarly, a steady increase in $DMSP_p$ was observed from day 1-10, which decreased from day 11-13 and again started increasing from day 14-18. The first peak of $DMSP_p$ (day 1-10) could be attributed to the rapid growth and reproduction of cells, whereas the second peak (day 14-18) may be due to the combined effect of growth and reproduction of new cells and ageing and lysis of parent cells. As seen from $DMSP_d$ and $DMSP_p$ plot (Fig. 2), in the early stages, DMSP remains mainly intracellular in the healthy cells, with the increase in extracellular fraction during the stationary and senescent phase which is also reported by Laroch et al., (1999); Zhuang et al., (2011).



Fig. 2a. Temporal variation of DMSP_t in *Skeletonema costatum* during light stress experiment stress experiment

Fig. 2b. Temporal variation of DMSP_d in *Skeletonema costatum* during light stress experiment stress experiment.



Fig. 2c. Temporal variation of DMSP_p in *Skeletonema costatum* during light stress experiment stress experiment.

Cell abundance normalized DMSP_p concentration calculated to examine per cell variation in DMSP production (cellular DMSP) is shown in figure 3. The cellular DMSP in *S. costatum* run varied between 0.08 and 0.59 pg S cell⁻¹. Based on cell bio-volume and per cell concentration of DMSP, it was observed that the marked growth phase of the culture with two distinct generations of cells, mature cells (38 - 49 μ m³), recorded till the 9th day of the growth phase followed by younger cells having a smaller cell size. Build-up of cellular concentration of DMSP was recorded at the late exponential to mid-stationary growth phase (days 6-10). Rapid reproduction of cells was observed from day 9 – 15 (Fig. 1a), having smaller bio-volume (Fig. 3) signifying new generation of cells. Thus, the smaller cells with higher surface-to-volume ratio drive the need for osmoregulation and in turn the production of DMSP. The maximum concentration of cellular DMSP (0.59 pg S cell⁻¹) was observed on day 10 during the culture run. Keller (1989), reported many of the smaller forms produce high concentration of DMSP per cell amounted to 0.22 ± 0.17 pg S Cell⁻¹. The recorded per cell concentration of DMSP in the present study was lower than 0.50 pg S Cell⁻¹ recorded by Keller et al., (1989) for *S. costatum*.



Fig. 3. Temporal variation of cellular DMSP and bio-volume in Skeletonema costatum during light stress experiment

3.3 DMSP, DMS and Chl a concentration at different growth stages

DMS concentration showed an increasing trend from lag to the stationary growth phase, which then remained constant from the stationary growth phase to the senescent phase. DMS concentration reached a maximum of 56.6 nM on day 7. By analyzing the data of DMS and Chl *a*, it was observed that there was a time lag between the peak of DMSP_t and DMS concentration, which could be due to the availability or breakdown of a small fraction of DMSP to DMS in healthy cells (Zhuang et al., 2011). During the culture experiment, DMSP_t concentration showed a similar trend with cell densities and remained relatively high at the late stationary and senescent phases (Fig.4). Recorded high DMS concentration in lag and the exponential growth phase may be attributed to light stress, which remained constant after changing the sampling time (culture flask, SF-2 at 9:15 AM and SF-1 at 9.45 AM, Fig. 5b & 5a). Observed high per cell concentration of DMS and DMSP on the first day of the culture experiment may be due to the carryover during transfer or inoculation of the culture and favourable metabolism in the fresh culture media (Zhuang et al., 2011).



Fig. 4. Temporal variation of DMS and $DMSP_t$ in *Skeletonema costatum* during light stress experiment

3.4 The effect of light on DMS production (experimental study)

The effect of light stress on DMSP and DMS production in *S. costatum* culture was investigated, wherein non-axenic cultures were grown in three flasks at 1000 lux light intensity using f/2 media. Varying concentration of DMS and DMSP was recorded in all three experimental culture flasks (Fig. 5). Sizable variation in DMS concentration was observed in SF-1 (10.0 – 113.0 nM), a flask that had undergone initial light stress on exposure to the start of 12 hour light cycle, thus possibly producing high DMS compared to the other two flasks acclimatized by the time they were sampled. This was confirmed by swapping the sampling time of the experimental flasks. To check whether the initial light exposure is playing a role in the production of DMS, the second culture flask (SF-2) was sampled first during the subsequent sampling (the next day), i.e. when the light was just turned on at the beginning of the 12 hour light cycle and tested for DMS/P. Interestingly, during this sampling, the second flask (SF-2) was observed to produce high DMS (160.0 nM) confirming the role of initial light exposure on DMS production. On the other hand, culture flasks sub-sampled during the acclimatised phase and dark phase were observed to produce very low levels of DMS, ranging from 3.0 - 10.0 nM generally recorded in the acclimatized culture flasks is SF-2 and SF-3. No such abrupt change was observed in the DMSP₁ in the three flasks.



Fig. 5 (a, b, c). Temporal variation of DMS, DMSPt and Chl *a* in *Skeletonema costatum* experimental culture flasks. SF-1, SF-2 and SF-3 are the culture flakes in triplicate.

These findings gave a clue that light intensity may act as a stress factor for the phytoplankton (*Skeletonema costatum*) inducing/making the algal cell produce/release more DMS under these conditions. Under stress conditions, the maximum DMS concentration of 160.0 nM was recorded during the growth phase (7th day) of the culture in the SF-2 flask. Whereas, the highest concentration recorded during the light acclimatized condition was only 10.0 nM of DMS. These results prompted us to explore the effect of daybreak in natural samples.

3.5 The effect of light on DMS production (laboratory and field study)

To understand the experimental data, that cell exposure to light acts as a stress on phytoplankton, studies were conducted in the laboratory on culture samples of diatoms and dinoflagellate, and on natural samples from the coastal and open Arabian Sea. In addition, an environmental time-series study was also carried out at the ASTS station (17°N 68°E) in the central Arabian Sea. The study

revealed a strong influence on the exposure of culture and natural samples to light (Table 1 and Fig 6). In the laboratory light exposure experiment on dinoflagellate samples, the DMS concentrations increased from nearly double to seven times, whereas in the case of diatoms, it increased from nearly double to fourteen times when compared to the samples before exposure to light. This was despite the fact that the cells were growing under different growth stages (Table 1, n = 16, p = 0.001). Similarly, the natural samples from the Arabian Sea also showed an increase from one and a half times to nearly thirteen times when compared to the dark samples. The above results suggest a strong influence of light (both natural and artificial) on DMS production. During the two-day environmental time-series study in the Arabian Sea, the light intensity, Chl a, DMS and DMSP_t ranged from 0.3 -88.9 mW Cm⁻², below detection level $-0.8 \ \mu g \ L^{-1}$, $0.3 - 25.2 \ nM$ and $0.4 - 69.2 \ nM$, respectively (Fig. 6). The 2-day time-series study also revealed that the light stress on phytoplankton resulted in DMS production. A peak in DMS concentration early in the morning soon after the sunrise (6:30 AM) was observed on both days, which could be due to the shock or light stress experienced by the organisms (Fig. 6). Since the organisms were adapted to the dark phase (night), the morning solar radiation acts as a stressor that induces DMS production. Following the morning DMS peak, high DMS concentrations were also observed during the afternoon, which may be attributed to phytoplankton productivity as seen with an increase in Chl a concentration. On the other hand, the high DMS at late night (~2 AM) may be attributed to the combined action of zooplankton grazing and grazing stress (Neale et al., 2003; Harrison and Smith, 2009; Galí and Simó, 2015; Royer et al., 2016). Phytoplankton coping with light stress is one hypothetical explanation for induced DMS production in phytoplankton by sunlight (Sunda et al., 2002). The availability of sunlight not only controls the phytoplankton activity fuelling photosynthesis but also causes light stress and photoinhibition (Neale et al., 2003; Harrison and Smith, 2009). While some studies (Slezak and Herndl, 2003; Darroch et al., 2015) have shown an increase in cellular DMSP concentration on exposure to PAR and UV radiation, others have shown a loss of DMS to photo-degradation on exposure to UVB, UVA and PAR (Xu et al., 2021). Most of the earlier studies involving DMS variability in the natural environment have mainly focussed on DMS cycling in relation to UV radiation and the mixed layer depth, which has shown high positive correlation among these variables (Simo and Alio, 1999; Toole and Siegel, 2004; Valina and Simo, 2007). Toole et al. (2006) observed a nine-fold increase in the DMS loss rates due to photolysis by UV exposure, however these changes were not correlated with the light dose during the incubations which they attributed to light-mediated DMS production. In a Lagrangian time-series studies conducted in the Mediterranean Sea, Royer et al. (2016) have discussed the influence of solar radiation on DMS cycling on short-term, sub-daily to weekly basis. They have shown the DMS increase observed daily between noon and afternoon is connected to

biological production, and the overall seasonal increase in DMS is connected to the gradual increase in solar radiation during sunny calm days. However, they did not report an increase in DMS concentration as observed in the present study. An increase in DMS production on sudden exposure to light may also be the response by phytoplankton cells to help maintain homeostasis (Stefels, 2000) or against antioxidants (Sunda et al., 2007). This is the first report connecting DMS production to light stress or initial light exposure wherein the laboratory observations were upheld by field studies. While this initial report indicate a strong influence of the first light exposure to DMS production, further studies are needed to unravel its potential impact on the DMS budgeting and ecological significance.

		DMS (nM)	
		Dark phase/	
		period	Initial exposure to
Laboratory culture (growth phase) / Natural field sample		measurements	light measurements
Dinoflagallates			
	G. instriatum (Exponential growth phase)	13.4	24.0
	G. instriatum (Exponential growth phase)	6.1	23.0
	G. instriatum (Exponential growth phase)	4.3	15.2
	G. instriatum(Stationary phase)	14.6	106.1
Diatoms	S. costatum (Exponential growth phase)	6.4	12.4
	S. costatum (Exponential growth phase)	3.1	9.3
	S. costatum (Exponential growth phase)	6.1	15.3
	S. costatum (Exponential growth phase)	5.2	23.6
	<i>S. costatum</i> (Stationary phase)	9.2	28.4
	S. costatum (Stationary phase)	5.8	31.5
	S. costatum (Stationary phase)	7.0	38.7
	C. wailesii (Stationary phase)	3.8	53.6
rabian Sea	DonaPaula Jetty (15°N 73°E)	8.4	13.6
	(SSD033, 17°N 68°E)	2.2	27.5
	(SSD061, 15°N 64°E)	4.2	19.0
٩	(SSD061, 21°N 63°E)	2.5	11.1

Table 1: Response of culture and natural samples to initial exposure to light (n = 16, p = 0.001)



Fig. 6. Variation of light, Chl *a*, DMSPt and DMS during a time series experiment on board RV Sindhu Sadhana (SSD-033). The shaded region in the graph represents dark period

4. Conclusion

The laboratory culture run on the diatom *Skeletonema costatum* has shown that build-up of cellular DMSP occurs during the late exponential to mid-stationary phase when the cells are actively dividing resulting in large cell numbers with smaller biovolume pushing the need for higher DMSP production for osmoregulation. Importantly, this study also revealed that the exposure to light after the dark phase acted as a stress on the algal cells, which was reflected by elevated levels of DMS in the culture flasks. This study was also upheld by data from experiments conducted, both in the laboratory and in natural samples, with further indications coming from a field time-series study, where, a peak in DMS concentration was observed during the sunrise confirming early morning light stress on phytoplankton inducing DMS production. As sunlight is one of the factors responsible for DMS production in the surface layer of the ocean the finding from this study should be researched further to understand its impact on DMS flux calculations and budgeting.

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Author contributions : BRN, MUG and DMS conceptualised the idea and the methodology, BRN executed the laboratory and field experiment and carried out the analysis/processing of samples. BRN, MUG and DMS wrote the manuscript.

Conflict of interest

The authors listed below have no conflict of interest to declare. All authors have seen and agreed with the content of manuscript.

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Data availability

Data generated during the current study will be provided on reasonable request.

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Figure Legends.

Fig. 1a. Temporal variation of Chl *a* and cell abundance in *Skeletonema costatum* during the light stress experiment

Fig. 1b. Temporal variation bio-volume in Skeletonema costatum during the light stress experiment

Fig. 2a. Temporal variation of DMSPt in *Skeletonema costatum* during light stress experiment stress experiment

Fig. 2b. Temporal variation of DMSP_d in *Skeletonema costatum* during light stress experiment stress experiment.

Fig. 2c. Temporal variation of DMSP_p in *Skeletonema costatum* during light stress experiment stress experiment.

Fig. 3. Temporal variation of cellular DMSP and bio-volume in *Skeletonema costatum* during light stress experiment

Fig. 4a. Temporal variation of DMS in Skeletonema costatum during light stress experiment

Fig. 4b. Temporal variation of DMSPt in Skeletonema costatum during light stress experiment

Fig. 5 (a, b, c). Temporal variation of DMS, DMSP_t and Chl *a* in *Skeletonema costatum* experimental culture flasks. SF-1, SF-2 and SF-3 are the culture flakes in triplicate

Fig. 6. Variation of light, Chl *a*, DMSPt and DMS during a time series experiment on board RV Sindhu Sadhana (SSD-033). The shaded region in the graph represents dark period