

Chapter 5

DISCUSSION

Dimethylsulphide (DMS) and Dimethisulfoniopropionate (DMSP) are the most abundant forms of organic sulfur compound in the ocean. Since 1970, DMSP has generated much interest because of its role as a precursor of DMS, a regulator of climate (CLAW hypothesis) (Lovelock et al. 1972; Charlson et al. 1987; Andréa, 1990). The main source of DMS in the ocean is phytoplankton, which uses DMS precursor DMSP as osmolyte. The production (Source) and utilization (Sink) of DMSP is one of the major processes that determine the potential amount of DMS that can be emitted to the atmosphere, and therefore the distribution of DMSP in the marine environments is of interest. However, only a few studies have been carried out on the influence of biological parameters in conjunction with environmental parameters on DMSP in shallow and coastal ecosystems. Further, studies related to DMS and DMSP in tropical waters are sparse.

For studying the temporal changes of photosynthetic related parameters such as DMSP and DMS, an environment with stable conditions are required. The choice of the study site is critical. A site that subdues the hydrographical influences and accentuates the physico-chemical variability had to be selected in order to understand the temporal variation of these compounds and factors influencing it. Dona Paula bay is unique in this respect due to restricted exchange process with the estuary as can be seen from the hydrological parameters studied. It was observed that the abiotic variations were marginal during the diurnal observations. Salinity showed very little variation during the diel period. This observation demonstrates that

there was no marked change in surface water in the bay during the period of study. Although the hydrographic parameters did not show marked variations during the annual observation also, a characteristic signature of tropical monsoon was discernable in the bay. Such seasonality is a characteristic phenomenon of estuaries of India. The monsoon influence has been repeatedly emphasized in earlier studies on Zuari estuary (Qasim and Sen Gupta, 1981, Shetye et al.1995). The study site although situated on the north eastern part of the estuary, the environmental parameters were comparatively low ($[\text{NO}_3]_{\text{max}} = 9.7 \mu\text{M}$; $[\text{Chl } a]_{\text{max}} = 4.8 \mu\text{g l}^{-1}$) [algal abundance] $_{\text{max}} = 10^4 \text{ cells l}^{-1}$) than those reported in the estuary (D'Sousa et al.1981; Sardessai and Sundar, 2007). This may particularly be due to the sheltered nature of the bay, which prevents mixing leading to low levels of the environmental variations. Most of the variables appeared to be conservative. A decreasing trend in salinity from 35.7 to 19 occurred at the study site between May to October as a result of increased precipitation. The drop in salinity was not much compared to the estuary (Ram et al.2007 and DeSouza et al. 2003) in the present annual study. Thus the main factor responsible for the small temporal change in the bay was the fresh water influx due to precipitation during the monsoon season. Therefore the present work mainly looked at the factors responsible for DMS sink or source in relationship with biotic and abiotic variables rather than hydrographic characteristics of the Dona Paula bay.

Temporal variation of DMSP and DMS

Diurnal variation

DMSP concentrations showed a diel variation with low concentration of 15.2 nM at night compared to that of the day (38.5 nM). Similar trend in the diel distribution of DMSP has been observed in other regions (Belviso et al. 2000 a; Yang et al. 2008) also. This diel variation was not due to the tidal influence as only one peak of DMSP synchronized with the ebb tide of the day and not with that of the night (Fig 3 in materials and method). The trend of DMS was similar to DMSP with low concentration at night. However, there was a decrease in surface DMS from 11:00 -17:00 hr. This might have been caused by photo-oxidation as solar insolation was high during this time period. A recent study indicates that under typical surface irradiation conditions, DMS photolysis is always more rapid than bacterial consumption. Slezak et al. (2001) has found that under surface irradiance condition, microbial removal of DMS (P) was inhibited up to 60% as bacterial sulphur demands could be less, under increased UV radiation. It could be deduced from the study that the variability in DMSP and DMS between day and night in Dona Paula bay may mainly be due to biotic factors. This was further confirmed as none of the abiotic variables correlated with PCA1. The overwhelming abundance of sulfate in seawater (≈ 28 mM) as compared to nitrogen (1-10 μ M), which is required in so many other physiological processes, may favor the production of sulfur containing compounds (Keller et al.1999). DMSP accounts for >50% of the total sulfur in some phytoplankton (Matrai and Keller, 1994).

Instantaneous concentration of DMS and DMSP is the net result of a tight cycling of production and consumption processes whose nature and rates may vary across the stratified water column (Wakeham, 1987; Kiene, 1992; Simo, 1995). Nonetheless, the importance of these to loss terms will vary as a function of irradiance, water clarity, temperature, phytoplankton occurrence, bacterial activity and sulphur demands (Slezak et al. 2001).

Being an algal product, diurnal DMSP production would be proportional to the diurnal photosynthesis process observed in algae. Recent finding by Simo' et al. (2002) concluded that the rate of synthesis is linked to photosynthesis, showing a diurnal signal of particulate DMSP. Similar observation was made by Zemmeling et al. (2006). They reported higher concentration of DMSP during the day and the increase was due to increased level of particulate DMSP. During the present diurnal study, it was observed that DMSP concentration varied with Chl *a* values significantly (Spearman's $r = 0.68$, $P < 0.01$). Moreover, as oxidative stressors such as increase in UV radiation, intracellular phytoplankton production of DMSP is expected to increase. Concurrently, Sunda et al. (2002) proposed that DMSP and its breakdown products function as apart of high-capacity cellular antioxidant. This could enhance accumulation of DMSP during the day. Hence, any trend observed in total DMSP was due to the particulate fraction. This might have influenced the increase in their concentration in the surrounding waters after the intensity of radiation decreased, as higher concentrations were observed late in the

afternoon. In addition, diurnal vertical migration of dinoflagellates can generate diurnal variation in DMSP concentration (Belviso et al. 2000; Merzouk et al. 2004). Thus, the concentration of DMSP depended on the producer. The increase in DMS (P) concentrations could also be traced to the occurrence of dinoflagellates, *Protoberidinium* sp and *Ceratium* sp during the time period. Jean et al. (2005) have reported that a dinoflagellate cell holds about five times more DMSP than a diatom cell. The intracellular DMSP content of *Ceratium* spp is found to be 9.8 pM cell⁻¹ and *Protoberidinium* spp to be 14.7 pM cell⁻¹ by them. Thus increase in DMSP values in Little Bay of Toulon was attributed to the occurrence of dinoflagellates. A similar trend was observed in St. Lawrence Estuary (Michaud et al. 2007) also. Studies have demonstrated that the DMSP containing dinoflagellates are active diel migrants (Belviso et al. 2000). Hence, the changes in planktonic composition affected the DMS (P) levels in these waters.

Absence of pronounced diel pattern in chlorophyll a concentration indicates that the DMSP may be siphoned off by the utilizers or low production than due to grazing effect (Erga and Skjodal, 1990). The concentration also depends on the utilizers as DMSP being a labile compound acts as a major sulphur source, a carbon source, and an osmolyte for heterotrophic microbes in the surface ocean (Kiene et al. 2000; Simó, 2001). It is rapidly cycled by microorganisms in the upper ocean with turnover times that typically range from 0.1 to 1 day. Kiene et al. (2000) found that the microbial conversion rate is dependent on

DMSP concentration and bacterial demand. They observed that at higher DMSP concentrations, a lower fraction of DMSP was assimilated, with a larger portion being converted to DMS as bacterial sulphur demands were satisfied. This could explain the peak observed in DMS concentration following DMSP hike. However, Michaud et al. (2007) have reported a simultaneous peak of DMSP and DMS in St. Lawrence estuary and suggested a rapid and regular cleavage of DMSP into DMS as well as the presence of active algal and / or bacterial DMSP lyase activity during the period. Nonetheless, the concentration in DMSP was tightly coupled to DMS concentration in Dona Paula bay and it was statistically significant (Spearman's $r = 0.68$, $P < 0.01$). Sciare et al. (2002) also reported a strong correlation between DMS and DMSP.

Very few studies have investigated the relationship between bacterial biomass and tidal regimes in estuarine area but no clear link has emerged. The viable population of cells utilizing DMSP varied with both pigments Chl *a*, (Spearman's $r = -0.71$, $P < 0.001$), and pheophytin (Spearman's $r = -0.68$, $P < 0.01$). During daytime DMSP hikes not only coincided with the producers but DMSP levels were found to vary (Spearman's $r = -0.90$, $P < 0.05$) with the abundance of retrievable number of bacteria though the abundance bacteria was low during the day. Slezak et al. (2001) observed that under surface irradiance condition, microbial removal of DMSP was inhibited up to 60% in the surface waters. Such a situation may also be applicable in the bay, though UV radiation was not measured during this study. Apart from

UV radiation, the abundance of DMSP utilizers was also higher during day, though difference between day and night was not significant. There is fragmentary information on microbial loop functioning at diel time scale from estuarine waters (Hyun et al.1999; Kolm and Andretta, 2003). Some authors have found that at night bacterial biomass is reduced by heterotrophic nanoflagellate predation (Wikner et al.1990; Solic and Krstulovic, 1998) which exceeds production. However, the heterotrophic nanoflagellates top down control on bacteria on a short time scale (from diel to daily) is disputed (Puddu et al. 2000; Iriate et al. 2003). Though the number of dinoflagellates was high in the night, the density of DMSP utilizers were not significantly low during the night, suggesting the absence of top down control on DMSPU. DMSP concentration and the bacterial groups were in first group PCA1 which accounted for 50% of variation (Table 8). Hence the photosynthetic production during the day coupled with a low utilization might have led to the build up of DMSP in Dona Paula bay. A more detailed study of the microbial population and its specific activity linked to UV would further improve our understanding of diurnal variation of DMSP.

Annual Variation

Although the hydrographic parameters did not show marked variations during the year-round observation, a characteristic signature of tropical monsoon was discernable in the bay. The monsoon influence has been repeatedly emphasized in earlier studies in the Zuari estuary (Qasim and Sen Gupta, 1981; Shetye et al. 1995). Though situated on the north eastern end of estuary, the environmental variables were

comparatively lower ($[\text{NO}_3]_{\text{max}} = 9.7 \mu\text{M}$; $[\text{Chl } a]_{\text{max}} = 4.8 \mu\text{g l}^{-1}$; [algal abundance] $_{\text{max}} = 10^4 \text{ cells l}^{-1}$) than those reported in the open estuary (D'Sousa et al. 1981; Sardesai and Sundar, 2007). Due to the bay's sheltered nature, the variables are constrained and lack of mixing could lead to low levels of the environment variables. The variability of DMSP concentration observed in the surface water of the bay covering an annual study was less than that of diurnal study. The monthly range of DMSP_t was from 0.7 – 15 nM and the annual mean concentration was $8.6 \pm 5.5 \text{ nM}$. Variation in DMSP range is in agreement with the earlier study along the west coast of India (Shenoy and Kumar, 2007) and was comparable to those reported from other coastal waters (Bates et al. 1987b; Michuad et al. 2007). Although studies in estuarine waters have concluded that several physico-chemical and biological factors are coupled to temporal trends in DMSP levels (Kiene and Service, 1991; Michaud et al. 2007; Sciare et al. 2002), the linear regression analysis of DMSP and abiotic variables in the present study showed no significant correlation. Low levels of DMSP were seen during March, and the ensuing months may be due high salinity as increase in salinity leads to subsequent retention of the compound within the cells. Temperature can also be a co-variant during this period as rate of DMSP disappearance from seawater is attributed as a function of temperature (Belviso et al. 1993; Kiene and Service, 1991). Interestingly, in these months our data showed variation in pH (DMSP and pH, Spearman's $r = -0.80$, $P < 0.001$) which increased concomitantly with high salinity. However, results from PCA analysis

showed the absence of abiotic variables from the first two components (Fig 35), indicating that the concentration of DMSP was not directly influenced by the abiotic variables presently studied.

Our data set could not trace annual variations in DMSP to phytoplankton density except during the monsoon months. The DMSP levels were high during monsoon months. Similar high values were observed for Chl *a* and phytoplankton density. Both their values showed a peak in July ($4.8 \mu\text{g l}^{-1}$ and $2 \times 10^4 \text{ cells l}^{-1}$ respectively) may be due to the increased nutrient levels. Since the vertical migration of dinoflagellates did not affect the bacterial load during diel study, same conclusions are drawn for annual study. Although DMS and DMSPp are ultimately from algae (mainly phytoplankton, except for some shallow coastal waters where macroalgae might also be important), it is uncommon to obtain a significant correlation between the concentration of DMS or DMSP and a biomass indicator such as Chl *a* (Leck et al. 1990; Wolfe et al. 1994; Kwint and Kramer, 1996; Townsend and Keller, 1996; Simó et al. 1997; Kettle et al. 1999) because DMSPp production is species-specific and DMS production is controlled by extracellular processes involving the entire planktonic community (Simó et al. 1997), such as zooplankton grazing (Leck et al. 1990; Christaki et al. 1996; Wolfe et al. 1997; Jones et al. 1998), microbial activity (Kiene and Bates, 1990; Kiene, 1992; Wolfe et al. 1994; Zimmer-Faust et al. 1996), and the release of algal lysis upon senescence or cell breakage (Wolfe et al. 1994). DMSP concentrations normalized to chlorophyll have been used to compare the ability of

different phytoplankton groups to synthesize DMSP. DMSP/Chl *a* ratio in the order of 100 nM μg^{-1} or higher are typical of oligotrophic open ocean waters, whereas those in the range of 3 - 30 nM μg^{-1} are generally characteristic of coastal, estuarine or ice-edge waters (Kiene et al. 2000). In the present study, the mean DMSP/Chl value was 4.1 nM μg^{-1} , suggesting the ability of plankton communities to produce or excrete DMSP into the bay (Fig. 24).

In our study changes in DMSP levels were often not reflected in changes in DMS though DMSP values were 1.4 fold high during the monthly studies. DMSP was higher in the showery months whereas DMS in the following months. DMSP and DMS were uncoupled annually in Tokyo Bay also (Niki et al.1997). Highest concentration of DMS was recorded in November (22.2 ± 2 nM). The dominance of DMS over DMSP in November and the fact that DMS increases despite the decrease in chlorophyll are indicative of post-monsoon algal decay. In this respect decreased chlorophyll levels during DMS peak concentrations observed in situ (Leck et al. 1990) and laboratory studies (Nguyen et al. 1988) indicate that the maximal DMS production took place during the declining phase of algal blooms. Also, earlier studies have reported prominent role by algal DMSP – lyase activity (Steinke et al. 1998). The previous study in the Zuari estuary has found the peak of DMS in July (Shenoy and Patil, 2003). From our limited knowledge about the area, the peak value of DMS in July 2001 coincided with a similar increase in salinity and this trend was visible throughout the study period. However, in our study the highest

concentration of DMS (22.2 nM) was higher than the previously reported concentrations for different ecosystems [e.g. Venice lagoon (16.3 nM), Moret et al. (2000), Danube estuary (11.9 nM) Amoroux et al. (2002), and Rhine estuary (10 nM); Sciare et al. (2002)]. It was interesting to observe that in April 2004 the DMS concentrations were higher compared to the DMSP levels. This trend recurred in November and next April. Normalized concentrations to chlorophyll have been used to compare the ability of different phytoplankton groups to synthesize DMSP (Fig. 24). This taxon specific dependence has been invoked as the main reason for the poor DMS vs chlorophyll correlations usually obtained, and for the variability of DMS (and DMSP ratios among different hydrographic and tropic regimes). The taxonomic composition of phytoplankton population remains as one of the best proxies for estimation of DMS/DMSP concentrations, although the reliability of these predictions is diminished by a host of other factors (Simo and Dachs, 2002). Clearly, the physiology and nutrient statuses of these populations also play a role (Keller et al. 2004).

Flux

Several results suggest that estuaries (Iverson et al., 1989; Cerqueira and Pio, 1999) could represent an important source of atmospheric DMS. The average flux observed during a diurnal observation was 4.8 ($\mu\text{M m}^{-2} \text{d}^{-1}$). These values are comparable with those in Venice lagoon (Moret et al. 2000) and greater than reported by Cerqueira and Pio, (1999) in a Portugal estuary in the subtropics. In our study, the highest flux was observed in the month of November corresponding to a peak

in DMS and differed from the previous investigation wherein the highest flux was in April (Shenoy and Patil, 2003). This may be due to fact the factors that govern DMS flux are not cyclic especially wind. The other factors affecting flux might be gas transfer velocities (K_w), which in turn are mainly dependent on wind speeds, water concentration of DMS, or the water temperature. During the diurnal experiments flux varied with DMS concentration (Spearman's $r = 0.66$, $P < 0.01$). Previous reports have pointed to water concentration of DMS as the main parameter controlling DMS flux in the estuary (Cerqueira and Pio, 1999). Monsoon ($11.4 \mu\text{M m}^{-2} \text{d}^{-1}$) values and post – monsoon ($10.5 \mu\text{M m}^{-2} \text{d}^{-1}$) values of flux were high compared to pre-monsoon ($6.1 \mu\text{M m}^{-2} \text{d}^{-1}$). Monsoon peak was due to high wind speed while in the post – monsoon, it was due to the combined effects of high DMS concentration and low temperatures. The mean flux value of $9.09 \mu\text{M m}^{-2} \text{d}^{-1}$ in the present study was in good agreement with model predicted by Andrea (1986) for the global coastal and shelf zones. The total emission for the bay was ca $0.1 \text{km m}^{-2} \text{yr}^{-1}$. Our estimates of sea to air flux of DMS indicate that the Dona Paula Bay is an important source of biogenic sulphur to the atmosphere.

Seasonal Variability

DMSP concentrations showed seasonal variations with prominent monsoonal and post-monsoonal peaks. It was two fold higher ($13.4 \pm 2 \text{ nM}$) during monsoon (June – Sept) compared to other seasons. Shenoy and Patil (2003) also recorded 1.6 times hike during the monsoon season. Similar high levels of DMSP during strong showers

have also been reported in the autumn in Toulon and Niel Bays (Jean et al. 2006). A clear seasonal pattern emerged in the bay during this study which was not so well marked in the mouth of the estuary during the investigation of Shenoy and Patil (2003).

Since DMSP is biogenic in origin, the factors that control the distribution and variation are expected to be those that influence the composition and activity of the biological community (Simo et al. 1997). In marine pelagic systems, a correlation has been found between Chl *a* and DMSP (Townsend and Keller, 1996), which is in agreement with the algal origin of this compatible solute. However, the correlation between algal biomass and DMSP is sometimes obscured by different levels of production (e.g. intracellular concentration) by different groups or species of algae, such as salinity or nitrogen availability (Turner et al. 1988; Iversion et al. 1989; Townsend and Keller, 1996).

The seasonal variations of DMSP_t concentration showed Chl *a* dependency. During monsoon, the increase in DMSP concentration was coincident with increase in phytoplankton density and Chl *a*. In July, Chl *a* and phytoplankton density showed peaks (4.8 µg l⁻¹ and 2 x 10⁴ cells l⁻¹, respectively). High DMSP/Chl ratio during monsoon season is indicative of the algal community's contribution to DMSP. The DMS/ Chl *a* ratio were high during these months. DMS/Chl reached a maximal value (3.6 nM µg l⁻¹) during pre-monsoon (Fig. 24) and was close to the values reported in Pearl River estuary by Hu et al. (2005) . The ratio in the bay were comparable to the values the Black Sea (3.6 nM µg⁻¹) and the Marmara Sea (3.95 nM µg⁻¹) but not to the

oligotrophic waters of Aegean ($39.4 \text{ nM } \mu\text{g}^{-1}$). The change in ratio depends on the species composition as a seasonal shift in ratio of DMS and DMSP/Chl *a* indicates a shift in algal community (Vogt et al. 2008).

Better evidence of the role played by dinoflagellates, diatoms and ciliates in the cycling of DMSP was obtained from an enumeration of these organisms than from the use of biomarkers (Belviso et al. 2000). Diatoms were the dominant group in the bay. Such dominance of diatoms has been reported in the west coast of India (Devassy and Goes, 1988; Jyothibabu et al. 2008). In Dona Paula bay also they were present throughout year with high numbers during monsoon season. Similar observation has also been made in an earlier study by Mitbavkar and Anil (2002). The production of DMSP is strongly dependent on phytoplankton species. DMSP is primarily synthesized by several phytoplankton groups, including dinoflagelletes, prymnesiophytes and diatoms (Yoch. 2002), and among these diatoms are poor producers of DMSP (Keller et al.1989). However, their contribution to DMSP pool may be significantly due to higher abundance as seen in Potomac estuary (Iverson et al. 1989) or when their intracellular level increases due to nutrient limitation (Bucciarelli and Sunda, 2003). During monsoon season not only their numbers were high but there was also a possibility of enhanced intracellular DMSP in diatoms due to low level of nutrient. Thus enhanced DMSP per cell and large number of diatoms may account for the high concentration of DMSP during the monsoon season. During the non-

monsoonal periods, dinoflagellate population increased and coexisted with diatoms. This shift in species may be due to the after effects of monsoonal rains similar to that observed in Toulon Bay wherein the autumn rains was accompanied by increased dinoflagellate abundance and a concomitant increase in DMSP levels (Jean et al. 2005). The eutrophication process changes the physical and chemical characteristics of sea water and induces a shift in planktonic community from diatoms to dinoflagellates in near shore and shelf waters (Mee, 1992; Lancelot et al. 1998). Increase in levels of DMSP due to eutrophication created by anthropogenic activity was observed in the Mediterranean Sea by Jean et al. (2006). As dinoflagellates are known to be prolific producers of DMSP (Keller et al. 1989; Jean et al. 2005), they could have contributed to DMSP pool as high numbers coincided with high concentrations of DMSP although there no significant relationship could be seen between dinoflagellates and DMSP in this study. Their presence and contribution to DMSP pool has been reported earlier by Shenoy and Patil, (2003) which was higher than that in the present study. This may be due to the proximity of their station to Marmagoa Port (MPT) which is located in the mouth of the Zuari estuary. The shipping channel site was more eutrophicated due to the input of nutrients as a result of periodic dredging exercises. This was corroborated by the high nitrate and phytoplankton biomass observed in the region ($[\text{NO}_3]_{\text{max}} = 11 \mu\text{M}$; Chl *a* levels ($[\text{Chl } a]_{\text{max}} = 11 \mu\text{g l}^{-1}$; [algal abundance] $_{\text{max}} = 10^6 \text{ cells l}^{-1}$) (Shenoy and Patil, 2003). Moreover, an occurrence of bloom was reported during their sampling

period. In the west coast of India whenever high concentration of DMSP was observed it was also associated with dinoflagellate blooms. A high value of 339 nM in November, 1999 and 419 nM in July, 2000 were reported off Candolim (15° 31' N, 73° 39' – 45' E) and at the mouth of the Zuari estuary respectively (Shenoy and Kumar, 2007). During the bloom years, the average concentration of DMSP in the coastal surface waters was ca 12 nM in March 2000. However, in the same month of the following year it was only 1.4 nM as no dinoflagellate bloom occurred during this year. The mean concentration of DMSP during this study was comparable to the earlier reported concentrations of DMSP in waters of the west coast of India during non-bloom period. In tropical waters unlike in temperate regions, occurrence of bloom is not a recurring phenomenon but rather a sporadic/ or episodic one (Bhat and Matondkar, 2004). The taxonomic composition of phytoplankton population remains as one of the best proxies for estimation of DMS concentrations, although the reliability of these predictions is diminished by a host of other factors (Simo and Dachs, 2002). Clearly, the physiology and nutrient statuses of these populations also play a role (Keller et al. 2004). In the shelf and estuarine waters, diatoms form a dominant group. In March dinoflagellates comprised about 20% of the total phytoplankton. There was an increase in the percentage of dinoflagellates (*Ceratium* spp., *Dinophysis* spp., *Noctiluca* spp., *Prorocentrum* spp). The second most abundant group, but with a substantially smaller biomass, were dinoflagellate species. These results show that most of the Chl a and

much of the DMSP are associated with the microplankton fraction that was dominated by diatoms of the genus *Chaetoceros* and *Nitzschia* (which accounted for 58% and 27% of diatoms respectively). The low specific DMSP measured is therefore predominantly a result of the abundance of DMSP poor organism, consistent with the abundance of diatoms during the cruise (Andreae et al. 2003).

Nevertheless, the taxa well known to produce high levels of DMS, prymnesiophytes (especially *Phaeocystis pouchetii*) were absent in our waters (DiTullio and Smith, 1995; Stefels et al. 1995; Turner et al. 1995; Kwint and Kramer, 1996). DMS/Chl *a* ratio for *Phaeocystis pouchetii* 58 – 78 nM μg^{-1} . Other than prymnesiophytes, dinoflagellates are known DMSP producers (Keller et al.1989, Townsend and Keller, 1996). Though they were not dominant group in the study area, species *Ceratium*, *Peridinium* and *Prorocentrum* were present and contributed to the DMSP pool . Although the diatoms are usually considered to be poor producers (Keller et al.1989; Stefels et al. 1995), there have been a few studies suggesting that the contribution of diatoms to the DMSP pool could not be overlooked (Levasseur et al. 1996). The phytoplankton community was dominated by diatoms of the genera *Nitzschia*, *Chaetoceros*, *Rhizosolenia* and *Thalassiosira*. Species from these groups contain little DMSPp (Dickson and Kirst, 1987; Keller et al. 1989; Matrai and Keller, 1994). These typical coastal diatoms were dominating in biomass in the bay year-round also contributed in the production of DMSP. And DMS/Chl *a* ratio were similar to that of other diatom dominated environment (2 -12 nM μg^{-1})

(Levasseur et al. 1996). Since the production depends on the species, the overall general low values and variation may be attributed to the fluctuation in the number and composition. In spite of their low number, there is ample evidence that among the phytoplanktons, dinoflagellates were the main producers of DMSP even though it is difficult to find a univocal relationship between dinoflagellates and DMSP in Dona Paula bay.

Role of bacteria

Bacteria play an important role in the cycling of sulphur within the marine environment, and the catalysis of DMSP to DMS is an important, well-established part of this cycle. Several studies have shown that DMSP alone can contribute 1 to 15% of the total bacterial carbon demand in surface waters. Marine bacteria play a major role in the transformation of DMSP via DMS and non – DMS producing degradation (Gonzalez, et al. 1999 & 2000; Kiene and Linn, 2000b) and this depends on the composition of the microbial community (Iverson et al. 1989). Heterotrophic bacteria not only breakdown DMSP into DMS (Kiene and Bates, 1990) but also translocate it directly as an osmolyte (Ciulla et al. 1997). Kiene et al. (2000) observed that the microbial conversion rate is dependent on DMSP concentration and bacterial demand. The enzymatic degradation of DMSP to DMS and acrylate has been observed in marine and estuarine bacteria (de Souza and Yoch; 1996; Kiene, 1990; Ledyard et al. 1993), fungi (Bacic and Yoch, 1998), and algae (Cantoni and Anderson, 1956; Kadota and Ishida, 1972; Stefels and Boekel, 1993). As the total count did not

show a marked gradient in their distribution, enumeration of DMSP utilizing bacteria in the water column may be crucial for understanding the role of bacteria in controlling DMSP concentration (Zemmelink et al. 2005). Though MPN method was useful for monitoring changes in DMSP utilizing population, it may underestimate the true bacterial population due to the selectivity of the media (Niki et al. 1997). The DMSP utilizing bacteria was of the order 10^3 cells l^{-1} in Dona Paula bay. This was about 1 to 10% of the heterotrophic bacterial population. In highly eutrophicated Tokyo Bay DMSP utilizing bacteria was an order of magnitude higher than that of the present study and utilizers varied from 4 to 44% of the heterotrophic bacteria (Niki et al. 1997). Surface waters of Caribbean Sea recorded ca 6% of the total heterotrophic bacteria (Visscher et al. 1992). The varying levels in DMSP and temperature correlated significantly (Spearman's $r = 0.68$, $P < 0.01$). Previous studies have pointed out the influence of temperature on dynamics of DMS (P) (Michaud et al. 2007; Kiene and Service, 1991). The link may be due to enhanced bacterial metabolism or through the complexity in the food web. Jean et al. (2005) have reported that temperature and photoperiod affects the production of DMSP. Thus in our study area DMS (P) is mainly governed by a suite of biotic parameters in sync with abiotic factors. Although sulfate is very abundant in seawater and bacteria have the capability to reduce it, so that they never get limited by this element, the use of available S in a reduced form would be energetically advantageous. Kiene and colleagues (1999) demonstrated the incorporation of the CH_3SH moiety

from DMSP into S-amino acids, so that it is very plausible that bacterioplankton are well suited to use DMSP, when it is available, to save energy for protein synthesis. Though, DMSP had a positive relation with DMSP utilizers, DMSP formation and excretion probably exceeded DMSP decomposition by bacteria, so that DMSP could accumulate during some months. The DMSP levels, DMSP utilizers and the measured bacterial parameters were grouped with the variables that highly correlated to PC1 in the PCA analysis. The bacterial density contributed significantly to the seasonal distribution of DMSP levels in the bay. The algal abundance was in the group of variables highly correlated with PCA 2 along with DMSP utilizers and the other bacterial groups. DMSP utilizing bacteria play an important but as yet unquantified role in the biogenic transfer of sulphur from the ocean to the atmosphere.

In this bay, the bacterial transformation of DMSP is an important factor determining its concentration, though during certain months changes in species composition and phytoplankton density also contributed to its concentration in the seawater. This study highlighted the coupled link between DMSP, phytoplankton and bacteria in exerting a control over DMSP concentration in Dona Paula bay.

In summary, the bay is influenced by the monsoonal regime. During the sw monsoon season, diatoms were identified as the major producers of DMSP in Dona Paula bay whereas during the non-monsoon seasons, dinoflagellates also contributed significantly to the

DMSP pool. DMSP utilizers were also responsible for variability in DMSP concentration. However, further studies on the rate of consumption and cellular DMSP stock of algae present in Dona Paula bay will help to separate physiological processes from the effect of trophic interaction on DMSP dynamics.

Diversity of DMSPU

Understanding the factors controlling DMS production in the marine environment relies on not only knowing the abundance but phenotypic diversity, and physiology of the microbes involved in this process. Marine bacteria play a role in the transformation of DMSP via DMS and non-DMS producing degradation (Gonzalez, et al. 1999 & 2000; Kiene and Linn, 2000a) and depend on the composition of the microbial community (Iverson et al. 1989). Probably, taxonomic composition is one of the factors influencing the extent of DMSP assimilation by bacterioplankton assemblages, thus influencing the DMS production. Therefore, knowing the diversity of the population involved in tropical waters is essential to understanding the global sulphur cycle. One of the current challenges in marine microbial ecology consists of identifying the phylogenetic groups that mediate key biogeochemical processes that are regulated over various temporal and spatial scales (Vila et al. 2007). Recent studies have suggested that, although DMSP-S assimilation is a widespread feature among groups of bacterioplankton, (Malmstrom et al. 2004 a; Vila et al. 2004) not all bacteria uniformly assimilate DMSPd (Malmstrom et al. 2004 a & b). In a year round study conducted at Blanes bay, both members of Alpha

proteobacteria and Gamma proteobacteria accounted for most of DMSP assimilating bacterial cells over the year (Villa et al. 2007). In the Arctic Ocean, a combined microautoradiography and fluorescence in situ hybridization (FISH) counts indicated that the bacterial assemblages belonging to alpha, gamma proteobacteria and bacterioidetes were utilizing ^{35}S - DMSP. In this study a description on identity of major groups of DMSP assimilating bacteria was done in order to assess the importance of bacterial assemblage, and their composition in controlling the fate of DMSP in the bay. Based on the scheme of Oliver (1982), bacteria growing on DMSP plates were identified as *Pseudomonas* sp (41%), *Xanthomonas / Flavobacterium* sp (15%), *Vibrio* sp (12%), *Moraxella* sp (2%) *Bacillus* sp (1%) and unidentified species (29%). The most abundant group was Gammaproteobacteria. Remarkably, Gammaproteobacteria contributed to total DMSP-S assimilating cells proportionally to their abundance over the year. In general, this group contributed equally to DMSP-S assimilation. Similar report on the prominent role of Gammaproteobacteria in DMSP-S assimilation was suggested by Vila et al. (2004). On a study conducted at North Inlet, South Carolina, US, the abundant group of DMS producers, obtained by plating dilutions on minimal DMSP medium, belonged predominantly to the Gamma subdivision and to a lesser extent, the Alpha subdivision of Proteobacteria. With the Gamma subclass representing 80 % of the isolates, many of the Gamma proteobacteria were closely related to the *Pseudomonads*; others were phylogenetically related to *Marinomonas*,

Psychrobacter or *Vibrio* sps (Ansedè et al. 2001). Similarly in our study, the majority of DMSP utilizing isolates were affiliated to Gamma subdivision of Proteobacteria. They were widely dispersed among various taxa with numerous isolates belonging to the genera *Pseudomonas*, *Moraxella* and *Vibrio*. (Ansedè et al. 2001). Moreover, Mou and colleagues (2005) found that 16S rRNA sequences belonging to the Gamma Proteobacteria group contributed 14 - 21% of the abundance of DMSP enriched communities in two of four samples collected from salt marsh waters. Although, DMSP utilization is a widespread feature among different groups of bacterioplankton (Vila et al. 2004; Malmstrom et al. 2004b) in temperate waters, members of two clades within Alphaproteobacteria group, Roseobacter and SAR11, when abundant seem to have a prominent yet not unique role in DMSP-S assimilation in the sea (Malmstrom et al. 2004a&b; Vila et al. 2004). Their high capability for DMSP metabolism was also reported in isolates of Roseobacter and SAR 11 taxa (Howard et al. 2006). These were absent in Dona Paula bay. The *Firmicutes* sps constituted a minority group with lowest percentage (1%). Recent studies with labelled ³⁵S- DMSP, have brought out the importance of other groups of bacteria in DMSP dynamics. The phylogenetic diversity of DMSP degrading bacteria is still largely unexplored. The limitations in culturing marine bacteria have long been known (Pace, 1996), so it is probable that only a small percentage of DMSP utilizers have been isolated in the present study. Our results also provide insights into DMSP utilization by poorly characterized marine taxa like the, *Alcanivorax*,

Bordetella and the *Bacillus* sp. Most members of the genus *Alcaligenes* and *Achromobacter* are found in the environment. Members of the genera *Bordetella* belonging to the family *Alcaligenaceae* occur in different ecological niches. However, sparse knowledge is available about the occurrence of *Bordetella* species in the natural environment. *Bordetella petrii* is the first member of this genus isolated from river sediment (von Wintzingerode et al. 2001). Among the isolated strains, reported in the literature, *Bordetella petrii* DSM12804 could degrade selenate (von Wintzingerode et al. 2001) and another *Bordetella petrii* strain could degrade naphthalene (Bianchi et al., 2005) in pure culture experiments. But the strains *Bordetella* sp. A-A20-1 and *Bordetella* sp. V-O7-1 could degrade PAH only in a mixed culture (Eriksson et al. 2003). The *Bordetella* strains , Strain_E3, DQ453688, Strain_F2, DQ453689 isolated by Wang et al., 2007 with closest match to *Bordetella petrii* AJ870969 were able to degrade 1,2,4-TCB. Ancede et al. (2001) reported that of all the DMSP utilizing bacteria isolated over a 5-year period from the salt marsh and estuarine water, only one isolate, *Alcaligenes* strain M3A, belonged to the Beta Proteobacteria. This is the first study reporting the strains belonging to the genera *Bordetella* utilizing DMSP. The type species, *Alcanivorax borkumensis*, was first described in 1998 to accommodate Gram-negative, halophilic, aerobic Gamma Proteobacteria that use aliphatic hydrocarbons as the sole source of carbon and energy (Yakimov et al. 1998). It belonging to the order *Oceanospirillales*. It has further been reported that members of the *Alcanivorax* genus are important marine hydrocarbon

degraders, since they are distributed in low numbers worldwide in coastal waters and sedimentary environments (Golyshin et al. 2003). Liu and Shao (2005) reported two bacterial strains isolated from the surface water of the Bohai Sea and deep sea sediment of the east Pacific Ocean to be *Alcanivorax dieselolei* sp. They grew on a restricted spectrum of organic compounds, including some organic acids and alkanes. Another DMSP-catabolizing bacterium (strain MWYL1), which had a 16S rRNA sequence 99% identical to that of *Marinomonas*, belonging to the order *Oceanospirillales*, a marine Gamma Proteobacterium has been isolated from root surfaces of the salt marsh grass *Spartina anglica* (Todd et al. 2007). This study reports the isolation and characterisation of *Alcanivorax* sps from the tropical waters for the first time. DMSP is an aliphatic compound that provides organic carbon along with sulfur (DMSP) to heterotrophic marine bacterioplankton (Kiene et al. 2000). Substrate utilisation studies which are discussed below showed its ability to use higher concentration of DMSP. Plausibly *Alcanivorax* sp has a role in the DMSP dynamics. Despite the limitations of using cultured bacteria to quantify their abundance in the natural environment, this study has nonetheless added to the accumulating knowledge of the diversity of the DMSP utilizing bacteria. Although not quantitative, and no doubt dependent on the isolation technique employed, this study has extended the DMS-producing characteristic into new phylogenetic territory.

Substrate Utilization by DMSPU

Identifying taxonomic patterns in metabolic ability is an important step for understanding drivers of DOC turnover in marine ecosystems, low taxonomic resolution (phylum, subphylum, class or family) limits access to the biochemical and genetic basis for DOC utilization. The estuaries serve as repositories of DOC, and its transformation is carried out by diverse communities of bacterioplankton whose rates and pathways, ultimately determine the form and amount of carbon and sulphur exported to the atmospheric and oceanic reservoirs (Moran et al. 1999). The organic osmolyte, DMSP is an important component of dissolved organic carbon (DOC). Given the importance of DMSP as a substrate in the temperate oceans (Kiene et al. 2000; Simó et al. 2002; Vila et al. 2004), it was equally important to understand its importance in tropical waters. In the bay, DMSP utilizers were positively correlated with DMSP concentrations and accounted for variation of these two compounds in the bay.

Detailed study was done on the substrate versatility of the utilizers. The bay isolates utilized various organo sulfur compounds and DMSP degradation products that occur as sulfur cycle intermediates in the marine environment. Taylor and Gilchrist (1991) reported that enrichments with DMSP selected bacteria that generated DMS, whereas MMPA enrichments selected organisms that produced methanethiol. Ancelescu et al.(2001) also found the same trend in the

isolates. However, the isolates from the bay showed the ability to grow on wide range of substrates other than the intermediates of DMSP (Fig. 38-42; Table 25, 28,31), with varying degree of growth thus reinforcing their prominent role as DMSP consumers. Recent studies have suggested that, although DMSP-S assimilation is a widespread feature among groups of bacterioplankton (Malmstrom et al. 2004a; Villa et al. 2004) not all bacteria uniformly assimilate DMSPd (Malmstrom et al. 2004 a& b). The growth of the strains on various supplied substrates established the substrate versatility of these strains which is an indication towards their adaptability. Among the phylotypes, the Gamma Proteobacteria was the most versatile in using these substrates for growth. Differential growth of marine bacterioplankton groups on specific substrates has been shown in several previous studies at the phylum/class level; Gamma Proteobacteria were shown to better utilize low molecular-weight DOC (such as amino acids and DMSP) than Beta and Gamma Proteobacteria and Bacteroidetes (Cottrell and Kirchman, 2000; Malmstrom et al., 2004a). Gamma Proteobacteria were shown to better utilize low molecular-weight DOC (such as amino acids and DMSP) than Beta and Gamma Proteobacteria and Bacteroidetes (Cottrell and Kirchman, 2000; Malmstrom et al. 2004a). Cultured members of Roseobacter clade have widespread abilities to degrade DMSP (Gonzalez et al.1999; Moran et al. 2003; Miller and Belas, 2004). *Silicibacter pomeroyi* strain DSS-3 is *Roseobacter* clade member, grows on a variety of organic acids, amino acids and other compounds, including DMSP, as sole

carbon sources (González et al. 2003). If these compounds are considered as representative of other DOC components, the utilisation of isolates indicate the possibility that they are able to transport and metabolize a wide range of compounds. If this is the case, possessing a variety of degradation pathways for commonly occurring substrates may be a successful ecological strategy in coastal seawater.

Utilization of DMSP at different physiological conditions

Bacterial utilization of DMSP is important as it can satisfy an important fraction of the bacterial carbon and sulphur demands in various marine environments (Kiene and Linn, 2000a&b). DMSP has been shown to support up to 13% of the bacterial carbon demand in marine surface waters (Kiene and Linn, 2000a; Simo et al. 2002). Recently, Vila et al. (2004) reported that 40% of the prokaryotes incorporated sulphur from [³⁵S] DMSP in the Northwest Mediterranean coast (Vila et al. 2004). The results of the enrichment experiments highlight the role of DMSP as a source of S and C. Enrichment culture studies of phototrophic purple bacteria from salt lakes demonstrated that DMS supported its growth by serving as an electron source for photosynthesis. Recent study has shown that DMSP turned over in the order of a few hours (Kiene et al. 2000). Heterotrophic bacteria exist as free or coexist with a diverse community comprising unicellular algae and other eukaryotic microorganisms in the marine environment. The spatial distribution of organic matter in these niches has established the heterogeneous structure of the micro-environment of bacteria. Based on the DMSP utilising capability and substrate versatility it was anticipated that the

organisms would exhibit other adaptations to living in association with marine phytoplankton or to the presence of DMSP at concentrations typical of phycospheres, that we assume to be intermediate to cytosolic and open-water concentrations. Dissolved DMSP concentrations in seawater range from 5 to 100 nM, but concentrations in algal blooms reach 1650 nM (Yoch, 2002) and cytosolic concentrations in phytoplankton range from 40 to > 500 mM (Yoch, 2002). DMS released by the breakdown of DMSP can be an environmental cue indicating the proximity of algae as a marker for their potential food supplies. Also the isolation techniques to date employed to isolates utilizing DMSP is based on growth in mill molar levels of DMSP as a criteria. These results must be viewed with caution because of the high concentrations tested.

The organic sulphur species are found year round in the waters surrounding the Indian peninsula. Hence the bacterial strains are bound to have specific properties as part of their capability to utilize DMSP. Chemotaxis to DMSP by pelagic bacteria is likely to be an important mechanism in establishing close interaction with the phytoplanktons at both the physical and physiological levels. Chemotactic experiment with representative isolates (Result Section 4.3.1.3) demonstrated that DMSP is a chemo-attractant for bacteria and utilize DMSP under a range of pH, temperature and salinity. The results of chemotactic experiments allows bacteria to detect nutrient gradients and motility is believed to serve as a method of approaching sources of food (Mitchell et al. 1999). Understanding this mechanisms

pertaining to bacterial chemotaxis towards DMSP may be of considerable significance both in still water and in turbulent flow environments. Most of the isolates in our present study were motile (90%). This provides motile bacteria with a distinct advantage over their nonmotile counterparts. Strains *Silicibacter* sp strain TM1040 and *Alcaligenes faecalis* M3A have in fact been shown to have chemotactic response to DMSP (Miller et al. 2004). The results with our isolates showed that they sensed and responded chemotactically to DMSP. These results reinforced that DMSP is a natural chemoattractant present throughout the year in our water.

Nonetheless, this study identifies bacterioplankton that are capable of increasing their activity in response to specific compounds typical of coastal DOC pools at high taxonomic resolution. These strains are capable of rapidly and broadly responding to the availability of DMSP in its environment and adapted to take maximum advantage of the presence of phytoplankton. Although the current experiment was conducted under laboratory conditions in batch culture in a rich medium, our observation of the gradation in affinity towards DMSP provide an interesting view on the ecological strategy of a cultured representatives of the successful bacterial groups (Beta, Gamma Proteobacteria and firmicutes) in the surface waters. Overall, the DMSPU of the bay showed the ability to degrade DMSP and related compounds is widespread across several major heterotrophic groups typically found in coastal seawater. It can be concluded from this utilization study that the taxonomic composition is one of the factor

influencing the extent of DMSP assimilation (sink) by bacterioplankton assemblages. This study highlights the importance of DMSP and how the composition of the producers and utilizers are the main players in the cycling of this compound in Dona Paula bay.