

Chapter 4

RESULTS

4.1 Ecological study

4.1.1 Diurnal study

4.1.1.1 Physico-chemical parameters

Tide

Changes in tide observed, during the diurnal study are presented in Figure 1a. High tide occurred during the day on both the days of sampling (16th and 17th December, 2004). The highest tide amplitude of 2.2 m was at 05:00 hr. One more was observed at 15:00 hr. The lowest tide of 0.83 m was at 21:00 and 09:00 hr. Overall, the tidal amplitude was high in the night ($1.29 \pm 0.5\text{m}$) compared to the day (Table 1).

Temperature

The temperature ranged from 27.8 to 30.2 °C with a variation of 2 - 3 °C during the diel experiments (24 hr). Temperature increased from 07:00 hr as the day progressed. (Fig.1b). The highest temperature of 30.2 °C was recorded at 15:00 hr while the lowest of 27.8 °C was recorded in the morning hours between 05:00 and 07:00 hr. The mean variability in the night was 28.6 ± 0.53 °C while it was 28.93 ± 1.03 °C (Table 1) indicating marginal mean variability.

pH

Diel variation in the pH was observed (7.6 – 8.5). The highest pH was recorded in noon hour (12:00 hr) (Fig. 1c). Generally pH was high during the day time (Table 1).

Wind

Wind speed showed lot of variation with higher speed during the day time. The highest wind speed (4.4 ms^{-1}) was at 11:00 hrs (Fig.1). The lowest wind speed (0.5 ms^{-1}) was at 15:00 hr.

Salinity

Salinity was more or less stable over the diel period (Fig.2a). Salinity was maintained at 36 except for minor variation to 34 during low tides. It dropped from 21:00 to 23:00 hr (36 to 34) and then stabilised to 36 (Fig. 2a). At 15:00 hr on both the days, salinity was 36 when tide height was $> 1.6\text{m}$.

Dissolved Oxygen (DO)

Unlike temperature and salinity, the DO displayed considerable variation. During the period of observation, DO levels ranged from 1.9 – 7.3 ml l^{-1} with high values at the night hours (Fig.2b). The highest value of 7.3 ml l^{-1} was observed at 23.00 hr. The mean value during the night ($6.2 \pm 1.00 \text{ ml l}^{-1}$) was higher than day ($5.1 \pm 1.3 \text{ ml l}^{-1}$) (Table 1).

Total Organic Carbon (TOC)

The TOC values ranged from 152 to 999.1 μ M. The highest values were recorded during night, at 21:00 hrs and the lowest value at 11:00 hr. During the noon hours the values fluctuated from 152 to 655 μ M (Fig. 2c). TOC showed diurnal variation with low values during the day ($414.5 \pm 201.86 \mu\text{M}$) compared to night ($539 \pm 298.19 \mu\text{M}$) (Table 1).

4.1.1.2 Organic sulfur compounds

DMSP

The DMSP concentration ranged from 3.9 to 84.3 nM (Fig.3a). The concentration increased as the day progressed. During the day, the values ranged from 19 to 42 nM except for a high value 84.3 nM at 17:00 hr. During the night, it fell from 27.8 to 3.9 nM. The lowest DMSP concentration was measured at 05:00 hr (3.9 nM). The mean value of DMSP was 2 times higher during day compared to night (Table 2). DMSP concentration decreased with temperature and an inverse trend was recorded with DO (Fig. 4).

DMS

The variations in the DMS concentration were relatively low compared to that of DMSP values (Fig 3). The DMS levels varied from 0.9 – 20.4 nM, with the lowest values measured at 01.00 hr and the highest at

19:00hr (Fig.3b). DMS recorded higher concentration in the day and lower concentration during night (Table 2).

Water to air DMS Flux

The average flux of DMS from the bay was $4.6 \mu\text{M m}^{-2} \text{d}^{-1}$. The highest flux was observed at 09:00 hr with a value of $13.32 \mu\text{M m}^{-2} \text{d}^{-1}$ (Fig. 3c). When the wind speed was lowest (0.5 ms^{-1}) during 15:00 hrs, the flux recorded was also the lowest ($0.61 \mu\text{M m}^{-2} \text{d}^{-1}$). The flux of DMS depended on concentration of DMS and wind. Scatter plot clearly showed the direct relationship between DMS concentration and wind (Fig.5 a & b). The flux was high during the day (6.00 ± 13.9) compared to the night (2.97 ± 3.30) (Table 2). The flux during the day was three times more than that of the night. This was confirmed as Spearman correlations was significant for DMS ($r = 0.687$) and wind ($r = 0.560$) (Table 3).

The interrelationship between the physico-chemical parameters, DMS and DMSP are tabulated in Table 3. Temperature showed a negative relationship with wind. Being a semidiurnal bay, salinity was influenced by tide as it was significant at <0.001 level. The concentrations of DMSP were depended on temperature and DO levels whereas DMS concentration was controlled by wind and DMSP concentration.

4.1.1.3 Biological parameters

Chlororophyll a (Chl a)

The diel variations of Chlororophyll a (Chl a) is presented in Figure 6 a. Chl a values ranged from 0.5 to 3.84 $\mu\text{g l}^{-1}$. The mean value during the 24 hr study period was $1.91 \pm 1.16 \mu\text{g l}^{-1}$. The highest concentration of 3.8 $\mu\text{g l}^{-1}$ was observed at 11:00 hr. Chl a was generally high during the day (2.62 ± 0.98) (Table. 4a). Normalized concentrations DMSP/DMS to chlorophyll have been used to compare the ability of phytoplankton to synthesize DMSP. The ratio of DMSP/Chl a between 17:00 to 19:00 hr was higher than rest of the day. In the case of DMS, high ratios were observed from 17:00 to 21:00 hr. As the ratios were low it indicates the ability of plankton communities' in producing or excreting DMSP in the bay (Fig.6b & c). Although DMS and DMSP were high during the day, no relationship could be seen between Chl a and these compounds (Fig.7a & b). Variables affecting chl a is tabulated in Table 4b.

Phaeopigment

Concentration of phaeopigments, ranged from 0.2 to 1.4 $\mu\text{g l}^{-1}$ and elevated values were noticeable during the late afternoon hours (Fig. 8). Phaeopigments values were higher during the day than at night

(Table 4a). Interestingly, phaeopigments showed relationship to DMSP (Table 4b).

Phytoplankton

Phytoplankton density ranged from 0.3 to 1.2×10^5 cells l^{-1} with an average of 0.8×10^5 cells l^{-1} . The high densities were observed during 07:00 hrs (1.2×10^5 cells l^{-1}) followed by 13:00 hrs (1×10^5 cells l^{-1}). The lowest counts were recorded (0.49×10^5 cells l^{-1}) at 15:00 hr (Fig. 9a). The abundance in phytoplankton density was high during the day time (Table 5). It showed a negative relationship to temperature ($r=0.9$, $p<0.05$)

Diatoms

The maximum density of diatoms was observed at 07:00 hr (1.1×10^5 cells l^{-1}). The density of diatoms (Fig. 9b) followed the same trend as the total phytoplankton and a significant relationship was observed ($r=0.9$, $p<0.05$). The average density of diatoms was 0.79×10^5 cells l^{-1} . Diatoms were higher during the day compared to night (Table 5).

Dinoflagellate

The dinoflagellate population exhibited a varied trend in the temporal distribution. The cell counts ranged from $0.1 - 1.1 \times 10^3$ cells l^{-1} . The highest value was recorded at 19:00 hr (Fig 9c). The dinoflagellate

density increased by dusk and the abundance was low during the day hours. Among phytoplankton, dinoflagellates contributed to about 0.5% of the total population. The dinoflagellate population was higher during the night compared to the day (Table 5).

Salinity did not show any trend with phytoplankton groups (Fig.10). Trend in distribution of the 3 groups of phytoplankton (diatoms, dinoflagellates and other algae) along with Chl *a* is presented in Figure 11. The maximum abundance of diatoms and other algae coincided with Chl *a* concentration. However, there was no correlation among these group with Chl *a*.

4.1.1.4 Bacteriological parameters

Total Counts

The total bacterial counts (TC) ranged from 1.3 to 5.8×10^9 cells l^{-1} (Fig.12 a). However, an order higher abundance was noticed at 17:00 hr (1.6×10^{10} l^{-1}). The average value of TC was 4.3×10^9 cells l^{-1} . TC was significantly correlated to salinity ($r=0.59$, $p<0.05$). The bacteria were more abundant during day with an average value of 5.6×10^9 cells l^{-1} than at night with a mean of 2.72×10^9 cells l^{-1} (Table 6).

Total Viable Counts

The total viable count (TVC) ranged from 1.3 to 2.8×10^8 cells l^{-1} (Fig.12b). The highest count was observed at 15:00 hr and the lowest count at 03:00 hr. The abundance of TVC depended on the total count at 03:00 hr. The abundance of TVC depended on the total count ($r=0.59$, $p<0.05$). Since TC was correlated to salinity and salinity to tide, the variation of TVC was influenced by these variables also ($r= -0.6$, $p<0.05$; $r=0.69$ $p< 0.001$). There were negligible variations in the abundance of TVC with a mean of 1.87×10^8 cells l^{-1} and 1.95×10^8 cells l^{-1} during night and day respectively (Table 6).

Colony Forming Units

Colony Forming Units (CFU) ranged from 0.6 to 2×10^6 cells l^{-1} and was 2 orders less than that of the TVC (Fig.12c). The temporal trend in CFU was different, where the highest count of 2×10^6 cells l^{-1} was recorded at 01:00 hr. During the day the mean value of CFU was 0.97×10^6 cells l^{-1} , whereas during the night, it was 1.74×10^6 cells l^{-1} (Table 6).

DMSP Utilizers and DMS Utilizers

The abundance of DMSP utilizers ranged from 0.3 to 1.1×10^3 cells l^{-1} . The population stabilized with a density of 1.1×10^3 cells l^{-1} from 01:00 to 07:00 hr (Fig. 13). The density of DMS utilizers ranged from 0.3 to 1.1×10^3 cells l^{-1} , with an average cell count of 0.56×10^3 cells l^{-1} .

DMS utilizers exhibited two maxima, at 03:00 and 15:00 hr. The temporal trend in abundance of DMSP and DMS utilizers were similar with both of them exhibiting relatively higher values during night hours (Table 6). No relationship was observed between the utilizers and their respective compounds (Fig. 14). The comparative trend in bacterial distribution during diel cycle is presented in Figure 15. The trend in the distribution of DMS and DMSP utilizers were similar to that of CFU. Spearman's correlation among biological parameters and DMSPU showed significant correlation between TVC and Chl *a* whereas DMSU showed relationship between phytoplankton and diatoms (Table 7).

4.1.1.5 Principal Component Analysis (PCA)

The Eigen values and Eigen vectors are tabulated in Table 8. The overall variance of all variables aggregated into 2 major groups (Fig 16). The first two components together explained 65.8 % of the variance. The first component explained 51.3 % of the variance. The variables highly correlated with the first axis (PCA1) were DMS, DMSP, TC, TVC and DMSPU. The first group highly correlated with PC1 reflects the influence of variables on DMSP and DMS during the study. The second axis, PC2 explained 14.6 % of the variance. The DMS was also grouped in PC2 along with DMSU, DMSPU and Chl *a*, DO, TOC, and wind.

4.1.2 Annual study

4.1.2.1 Physicochemical parameters

Temperature

Temperature varied from 20 to 29.5 °C, a variation of 5 - 8 °C (Fig. 17a). Over this period of one year the surface temperature showed 3 values greater than 25 °C, during the pre-monsoon months of March (26.5 °C), April (28.5 °C) and May (29.5 °C). The lowest temperature was observed in January (20 °C). Temperature exhibited seasonal variation with a mean value of 27.10 ± 3.07 in pre – monsoon, 22.25 ± 1.19 in monsoon and 21.88 ± 1.49 post- monsoon periods (Table 10).

pH

The pH fluctuated considerably and ranged from 7.5 to 8.2 the lowest in November and the highest in March. The pH values were relatively higher during the pre-monsoon months and averaged to 8.06 indicating no freshwater influence. The pH was low during monsoon months with an average of 7.63 ± 0.05 and 7.88 ± 0.22 during post-monsoon months (Table 10). The low pH observed during these seasons is characteristic of precipitation and freshwater mixing at the study site.

Wind

The highest wind speed (8.5 ms^{-1}) was recorded in July (Fig. 17c). The lowest wind speed (3.9 ms^{-1}) was recorded in October. The wind speed was more during the monsoon months (Table 10).

Salinity

During the study period, salinity ranged from 19 to 35.7 (Fig. 18a). Salinity was 34 or more during the dry periods. It dropped from 35.7 in May to 19 in June but gradually recovered during July. The lowest salinity in June was due to precipitation. After the monsoon, the salinity gradually increased to 35.7 in November and stabilized later at around 34. During the pre-monsoon the salinity averaged 34.63 ± 0.69 whereas during the monsoon and post-monsoon, the values were 24.25 ± 6.4 and 35.23 ± 0.82 , respectively (Table 11).

Dissolved Oxygen (DO)

The annual profile of DO showed considerable fluctuation (Fig. 18b). The highest concentration was recorded in of July and the lowest November. Intra seasonal variation in dissolved oxygen was less. The lowest value for DO was obtained in the post-monsoon season (November) and the highest during the monsoon season (July). The highest seasonal average was observed during monsoon with a value

of 4.48 ± 0.37 , whereas during pre- and post-monsoon the values were 3.94 ± 0.36 and 3.4 ± 0.29 (Table 11).

Total Organic Carbon

The TOC values ranged from 102 to 366 μM (Fig. 18c). The highest concentration was observed in September. Variation in concentration was not very large within each season. The highest TOC was measured during the monsoon months with a mean of 281.65 ± 86.74 μM . The lowest TOC values were determined during pre-monsoon month of April, and averaged 129.6 μM for this season. The average TOC value during post-monsoon was 201 ± 65.59 μM (Table 11).

Nitrate

The annual variation in nitrate concentration is shown in Figure 19a. The nitrate concentrations ranged from 0.49 – 9.7 $\mu\text{g at N l}^{-1}$ during the period of investigation. High concentrations were observed in August, September and October. The nitrate concentration was high during the monsoon season (5.1 $\mu\text{g at N l}^{-1}$). The nitrate concentrations were the lowest during the pre-monsoon season (2.9 $\mu\text{g at N l}^{-1}$) whereas during the post-monsoon season the mean value was 3.2 $\mu\text{g at N l}^{-1}$ (Table12).

Nitrite

Nitrite content showed the same trend as that of nitrate (Fig.19b). The nitrite concentration ranged from 0.2 to 2.7 $\mu\text{g at N l}^{-1}$ at the bay. Like nitrate, the nitrite values were high during the monsoon season with an average of 1.19 $\mu\text{g at N l}^{-1}$ (Table12). During the post-monsoon the mean values decreased (0.70 $\mu\text{g at N l}^{-1}$), but increased to a mean value of 1.02 $\mu\text{g at N l}^{-1}$ in the pre-monsoon.

Phosphate

The phosphate values ranged from 0.5 to 3.7 $\mu\text{g at P l}^{-1}$ (Fig.19c). High values of phosphate were recorded during the pre-monsoon months of February, March and May. The concentration in April '04 was low but in the following year (April '05) the concentration was the highest. The mean phosphate value was high in pre-monsoon season (mean 2.39 $\mu\text{g at P l}^{-1}$). During the monsoon season, the mean value was 1.35 $\mu\text{g at P l}^{-1}$ and was 1.02 $\mu\text{g at P l}^{-1}$ during the post-monsoon (Table 12).

4.1.2.2 Organic sulfur compounds

DMSP

Annual mean of DMSP at the study site was 8.6 ± 5.5 nM. DMSP levels recorded peaks in June (14.5 nM), August (15.8nM), November (13.2 nM) and December (15.1nM). It was low in March, April and May

(Fig. 20a). A profound seasonality was observed during the annual cycle (Table.13). DMSP values were four fold higher during monsoon (13.4 ± 2.09 nM) and three fold high during post-monsoon (9.96 ± 5.28 nM) compared to pre-monsoon period (2.80 ± 1.84 nM).

DMS

The annual mean of DMS concentration in the waters was 6.01 ± 6 nM. DMS was low throughout the study period except for a high value in November (22.3 nM) (Fig. 20b). The mean DMS concentration was relatively high during the post-monsoon season (9.46 ± 9.03 nM) compared to monsoon (4.2 ± 2.14 nM) and pre-monsoon seasons (4.37 ± 3.53 nM) (Table 13).

Flux

The mean value of the flux was $9.09 \mu\text{M m}^{-2} \text{d}^{-1}$ in the present study. The DMS flux from sea to air showed 2 peaks. The highest flux was observed in November ($24.7 \mu\text{M m}^{-2} \text{d}^{-1}$) corresponding to a peak in DMS (Fig. 20c). The flux was low in the months of January (2.2), February (2.0), May (4.7) and October (4.5). During these months both DMS concentration and wind speed affected the flux. Seasonal trend of flux is quite similar to that of the DMS concentration except in monsoon when there was a peak in flux but no corresponding peak of DMS concentration. Monsoon ($11.4 \mu\text{M m}^{-2} \text{d}^{-1}$) value and post-

monsoon ($10.5 \mu\text{M m}^{-2} \text{d}^{-1}$) value of flux were high compared to pre-monsoon ($6.1 \mu\text{M m}^{-2} \text{d}^{-1}$) (Table 13). Flux varied depending on wind and DMS concentration (Fig. 21). Mean flux of DMS for three seasons was computed and the total emission of biogenic volatile sulphur from the bay was found to be ca $0.1 \text{ km m}^{-2} \text{ yr}^{-1}$.

The significant interrelationships between all the physio-chemical parameters observed for a year are tabulated in Table 14. Temperature showed a positive relationship with phosphate. TOC level was negatively correlated to pH and temperature. The concentration of DMSP was depended on pH (negative correlation) and TOC whereas DMS was negatively correlated to DO and positively influenced by wind.

4.1.2.3 Biological parameters

Chlorophyll a

The chlorophyll *a* values ranged from 0.7 to $4.8 \mu\text{g l}^{-1}$. The high chlorophyll values of $4.8 \mu\text{g l}^{-1}$ and $4.7 \mu\text{g l}^{-1}$ were recorded in July and June respectively (Fig. 22a). The lowest value was recorded in March ($0.8 \mu\text{g l}^{-1}$). Considerable monthly variations were observed. Chl *a* concentration showed the opposite trend to salinity. In June and July when Chl *a* was high, salinity was low (Fig. 22b). Similarly, when Chl *a* was low in pre-monsoon months, salinity was high. However,

Spearman analysis did not show any significant relationship. Total nitrogen showed the opposite trend to Chl *a* (Fig. 22c). Spearman analysis showed significant correlation between Chl *a* and nitrate and nitrite ($r = -0.67$ and -0.72 respectively at $p < 0.01$). Chl *a* did not show any relationship with either DMSP or DMS as can be seen from the scatter plot (Fig. 23a,b). Normalization of DMS and DMSP with Chl *a* showed values less than 6, except in September for DMSP and in November for DMS. DMSP/Chl *a* ranged from 0.2 to 11.2 nM μg^{-1} (Fig. 24a). The DMSP/Chl *a* values were in August (11.2 nM μg^{-1}) and February (6.4 nM μg^{-1}). The lowest values were noticed in the month of March 0.2 nM μg^{-1} . The mean DMSP/Chl *a* value of 4.1 nM μg^{-1} reflects the plankton communities' ability in producing or excreting dissolved dimethylsulphide in the estuary. The DMS/Chl *a* values ranged from 0.3 to 7.67 nM μg^{-1} . DMS/Chl *a* reached a maximal value (7.67 nM μg^{-1}) in the month of November (Fig 24b). Monsoon period recorded the highest Chl *a* with a mean value of 3.19 $\mu\text{g l}^{-1}$ (Table 15).

Phaeopigment

The phaeopigments ranged from 0.19 to 1.56 $\mu\text{g l}^{-1}$. The highest concentration of phaeopigment was recorded in April '04 (1.56 $\mu\text{g l}^{-1}$) (Fig. 25). Phaeophytin pigments were relatively more during pre-monsoon season (Table 15), although the variations between the seasons were marginal.

Primary Productivity

The primary productivity values ranged from 14.5 to 204 mg C m⁻³ h⁻¹ (Fig. 26a), the highest being recorded in December. The productivity was low and registered values of 16.6mg C m⁻³ h⁻¹ and 14.5mg C m⁻³ h⁻¹ in May and June, respectively. However, primary production did not coincide with Chl *a* (Fig. 26b). During post-monsoon period, PP showed a wide range of variation (28 to 204.3 mg C m⁻³ h⁻¹). The seasonal trend showed the highest productivity during the post-monsoon season (93.80 ± 77.01 mg C m⁻³ h⁻¹) compared to pre-monsoon and monsoon seasons (Table 15). Spearman analysis showed significant relationship between PP and DMS (0.687 p at <0.01).

Phytoplankton

The phytoplankton density ranged from 0.7 - 20 x 10³ cells l⁻¹ and the density fluctuated during the study period (Fig. 27a). Its abundance was high in June (2 x 10⁴ cells l⁻¹) and the overall abundance was higher during the monsoon season compared to pre-monsoon values (Table 16). Like Chl *a*, the seasonal maxima for phytoplankton density was observed during the monsoon season with a mean density of 9.15 x 10³ cells l⁻¹. The variation in phytoplankton with total nitrogen is shown in Figure 28. As can be seen, there was inverse trend

between the two variables except during the pre-monsoon months. However, phytoplankton abundance was not parallel to that of Chl *a* except in the month of July and November (Fig. 29).

Diatoms

In the bay, diatoms contributed significantly to phytoplankton population in the year round study the highest density was observed in July ($19.6 \times 10^3 \text{ cells l}^{-1}$) and the lowest density was seen in May ($0.7 \times 10^3 \text{ cells l}^{-1}$) (Fig. 27b). Diatom density was correlated to phytoplankton density ($r=0.989$, $p<0.01$). They were abundant during monsoon season with a seasonal average of $8.99 \times 10^3 \text{ cells l}^{-1}$.

Dinoflagellates

The dinoflagellates were low throughout the study period (Fig. 27c). However, the number increased relatively in October to January ($0.5 \times 10^3 \text{ cells l}^{-1}$) and the highest density was observed in November ($6.9 \times 10^3 \text{ cells l}^{-1}$). During monsoon period, their representation was nil in the population. Their contribution ranged from 6 to 14% and 0.3 to 4% of the total planktonic population during post and pre-monsoon, respectively (Table 16).

Trend in distribution of the 3 groups of phytoplankton along with salinity is presented in Figure 30. As seen in the figure the abundance of dinoflagellates coincided with salinity ($r=0.772$, $p<0.001$).

4.1.2.4 Bacteriological Parameters

Total Count

TC ranged from $0.8 - 15 \times 10^9$ cells l^{-1} . TC was the highest during the month of October 1.5×10^{10} cells l^{-1} . The numbers were more or less constant, except for another minor peak in June (Fig. 31a). Among the various parameters, TC showed an inverse trend with nitrate concentration ($r = -0.67$, $p < 0.01$) (Fig. 32). The lowest bacterial density was observed during pre-monsoon season (mean 2.0×10^9 cells l^{-1}). The monsoonal mean was 2.97×10^9 cells l^{-1} , while during post-monsoon it was 6.82×10^9 cells l^{-1} (Table 17).

Total Viable Count

TVC ranged from $0.01 - 3.8 \times 10^8$ cells l^{-1} (Fig. 31b). TVC was the highest in October with a value of 3.8×10^8 cells l^{-1} . The trend in distribution followed that of TC. The mean TVC during monsoon and post-monsoon seasons were very similar but during the pre-monsoon season it was 10 folds lower (0.20×10^8 cells l^{-1}) (Table 17).

Colony Forming Unit

The lowest value was recorded in the month of April '04 (0.04×10^6 cells l^{-1}). The CFU was the highest in the month of September (7.9×10^6 cells l^{-1}) (Fig. 31c). The maximum density of CFU did not coincide

with that of TC and TVC densities. The mean densities were 0.24, 3.7 and 1.93×10^6 cells l^{-1} for pre-monsoon, monsoon and post-monsoon respectively (Table17).

DMSP and DMS Utilizers

The annual mean of DMSP utilizing population was 5.2×10^3 cells l^{-1} while that of DMS was 2.8×10^2 cells l^{-1} . The DMSP utilizers were the highest during September with a value of 1.2×10^3 cells l^{-1} (Fig. 33a). The DMSP utilizers were high in the monsoon months (mean 7.5×10^3 cells l^{-1}) whereas the DMS utilizers in monsoon season (mean 4.6×10^2 cells l^{-1}) (Table 18). The DMS utilizers also were high during September with a value of 9.5×10^2 cells l^{-1} (Fig.33b). The scatter plot between the utilizers and the compounds are shown in figure 34.

Interrelationship between bacterial variables and all measured parameters are tabulated in Table 19. Significant correlation were observed among the (TVC, CFU, & DMSP utilizers; $p < 0.0001$).

4.1.2.5 Principal Component Analysis (PCA)

The PCA analysis aggregated the variables into two major components, together explaining 71% of variation in annual study. The first group in the left was highly correlated with PC1, which reflected the influence of variables on DMSP during the study (Table. 20). The variables that highly correlated with first axis (PC1) were DMSP, TOC,

Chl *a*, DMSP utilizers, TVC, and TC which explained ca 54% of variations (Fig. 35). In year-round study NO₃ figured in PC2. The variables highly correlated with PC2 were DMSP utilizers, CFU, TVC, phytoplankton abundance, and nitrate, which explained 16.5% of the variability in the annual study. These variables indicated the influence of phytoplankton abundance on the bacterial population.

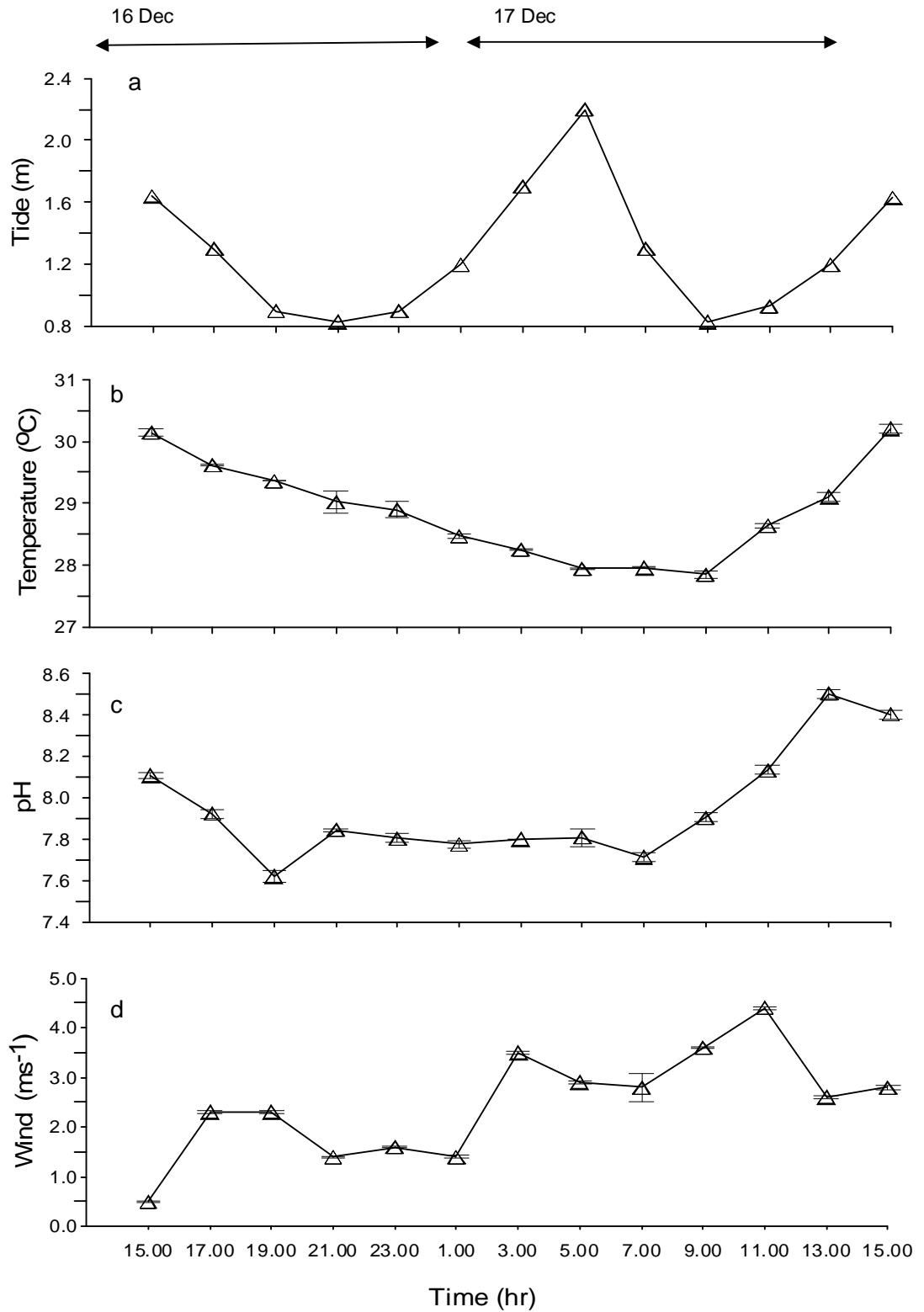


Fig.1 Diurnal variations of a) Tide, b)Temperature) pH and d) Wind at Dona Paula bay

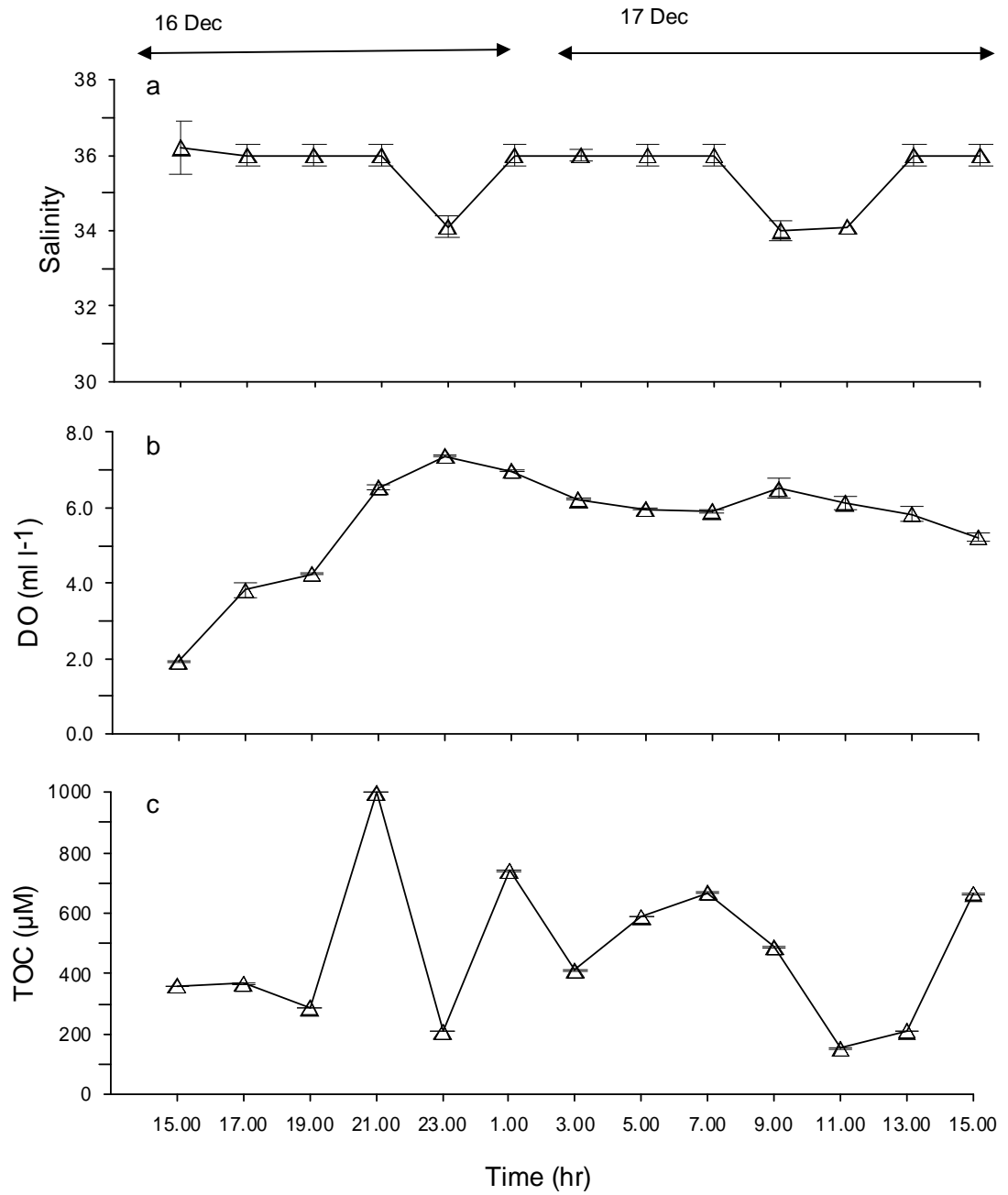


Fig. 2 Diurnal variations of a) Salinity, b) Dissolved Oxygen (DO) and c) Total Organic Carbon (TOC) at Dona Paula bay

Time	Tide (m)	Temperature (°C)	pH	Wind (ms ⁻¹)	Salinity	DO (ml l ⁻¹)	TOC (µM)
Day	1.26 ± 0.31*	28.93 ± 1.03	8.10 ± 0.28	2.70 ± 1.00	35.47 ± 0.97	5.19 ± 1.30	414.57 ± 201.86
Night	1.29 ± 0.50	28.6 ± 0.53	7.75 ± 0.07	2.18 ± 0.80	35.68 ± 0.78	6.22 ± 1.00	539.25 ± 298.19

Table. 1 Mean variability of Tide, Temperature, pH, Salinity, Dissolved Oxygen (DO) and Total Organic Carbon (TOC) at Dona Paula bay * (± SD)

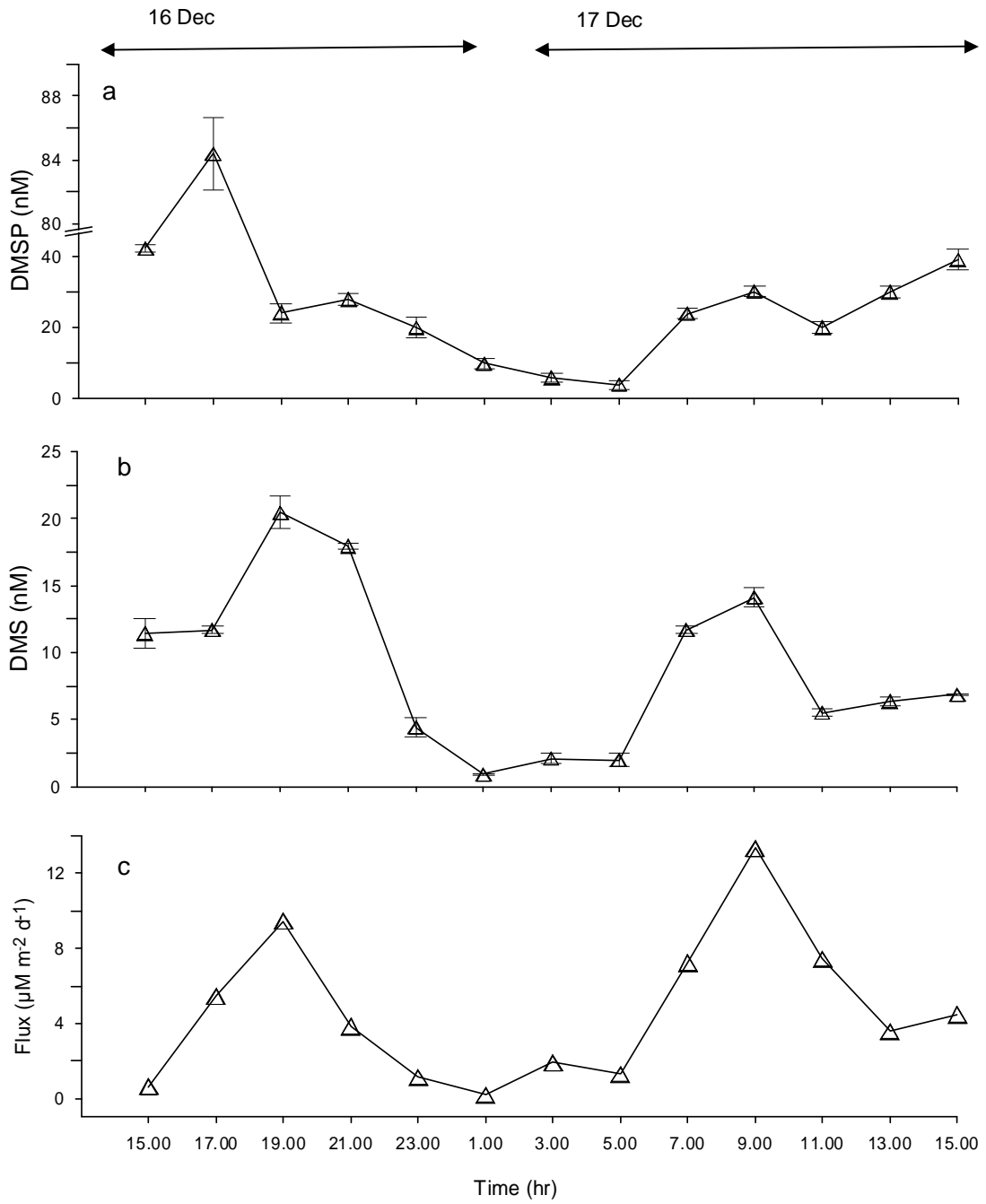


Fig. 3 Diurnal variations of a) DMSP, b) DMS and c) flux at Dona Paula bay

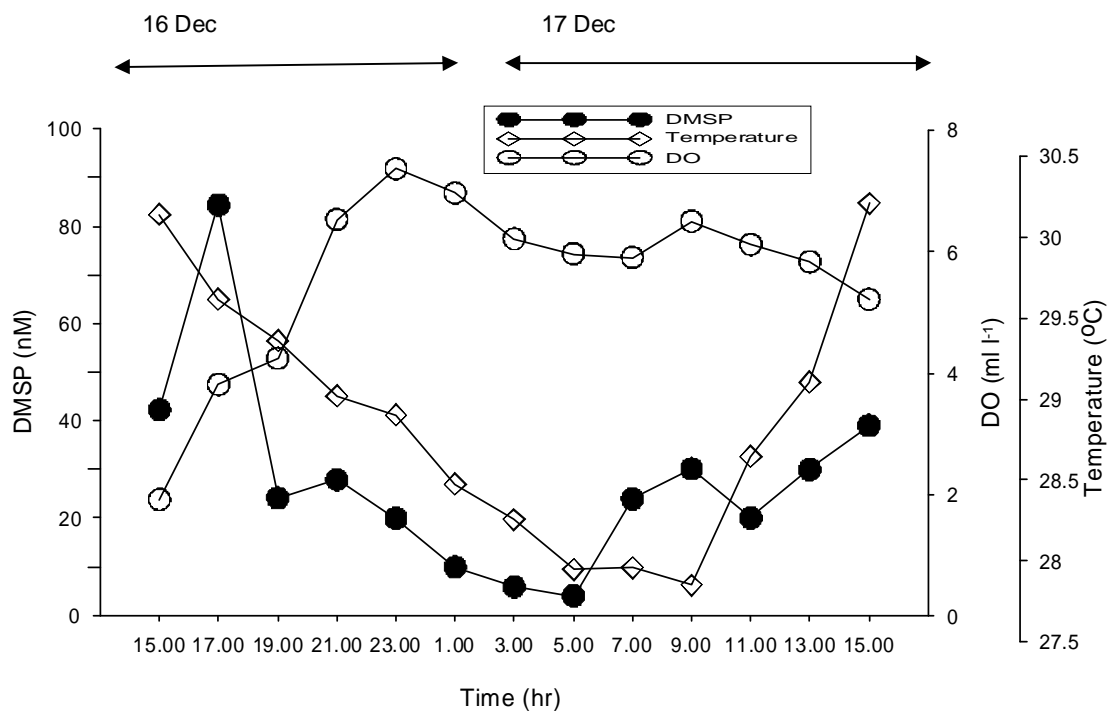


Fig. 4 Variation of DMSP with DO and Temperature during diurnal study

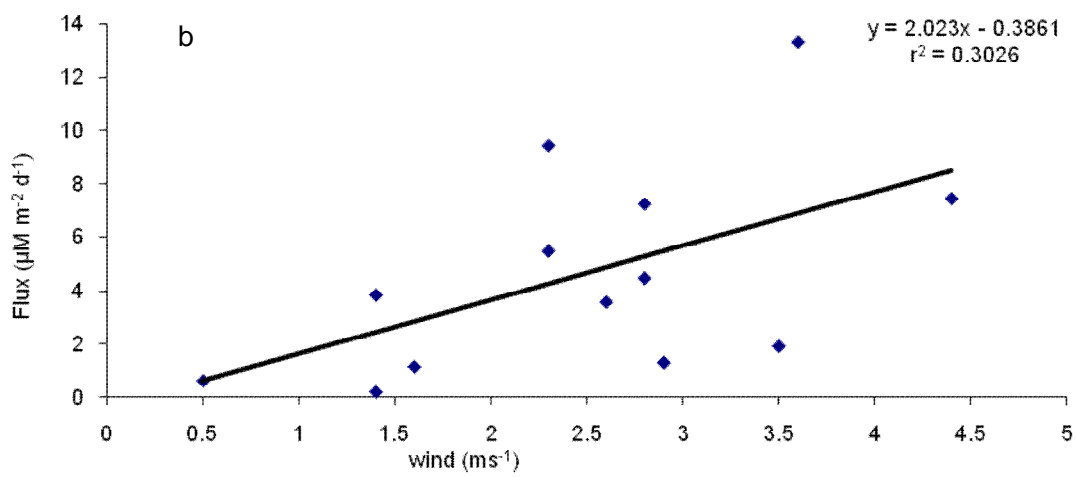
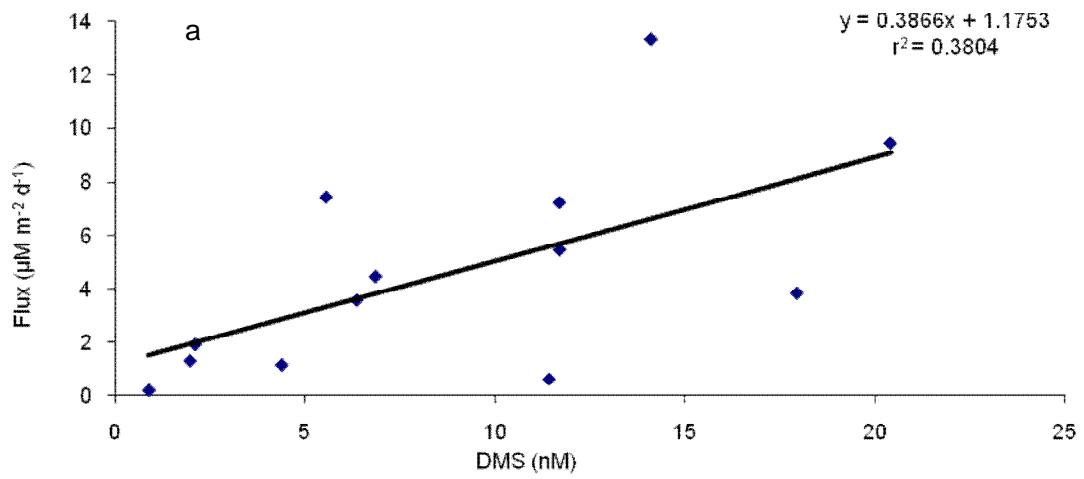


Fig. 5 a) Scatter plot of Flux versus DMS, b) Scatter plot of Flux versus wind

Time	DMSP (nM)	DMS (nM)	Flux ($\mu\text{mol m}^{-2} \text{d}^{-1}$)
Day	38.49 \pm 21.66*	9.68 \pm 3.34	6.00 \pm 13.90
Night	15.28 \pm 10.05	7.96 \pm 8.80	2.97 \pm 3.30

Table. 2 Mean variability of DMSP, DMS and Flux at Dona Paula bay

(\pm SD)

Variables	Spearman r	p value
Temperature & Wind	-0.578	< 0.05
Tide & Sal	-0.816	< 0.001
DMSP & Temperature	0.683	< 0.01
DMSP & DO	-0.615	< 0.05
DMSP & DMS	0.676	< 0.01
Flux & DMS	0.687	< 0.001
Flux & Wind	0.56	< 0.05

Table. 3 Spearman correlation coefficients of Physico - chemical variables during diurnal study at Dona Paula bay

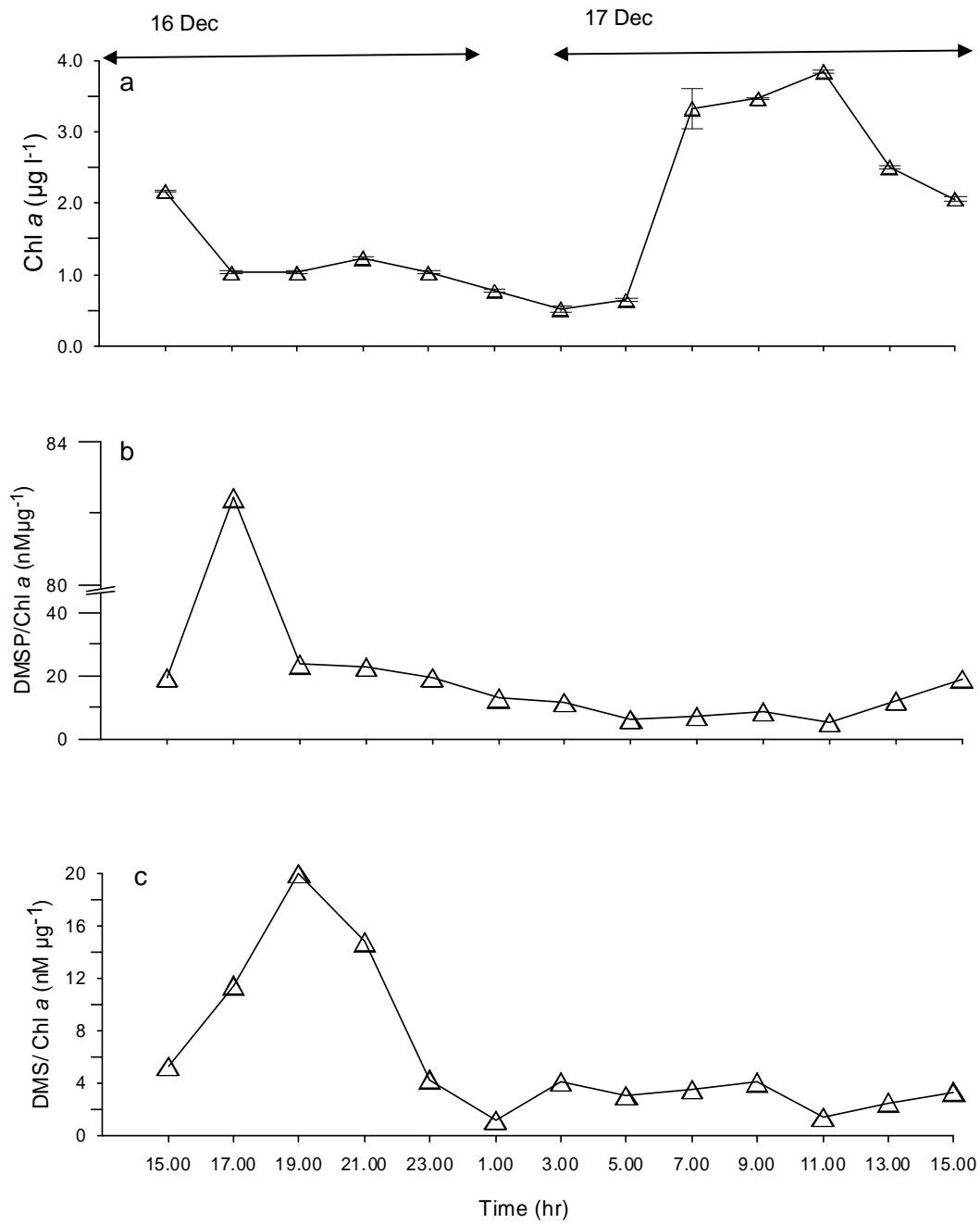


Fig. 6 Diurnal variations of a) Chlorophyll a , b) Ratio of DMSP/Chl a and c) DMS/ Chl a at Dona Paula bay

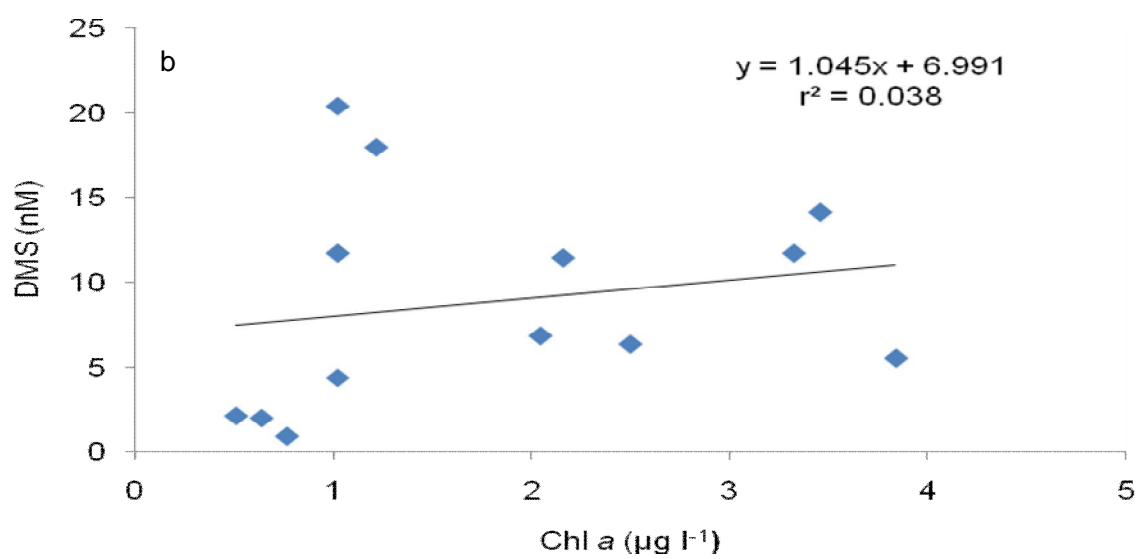
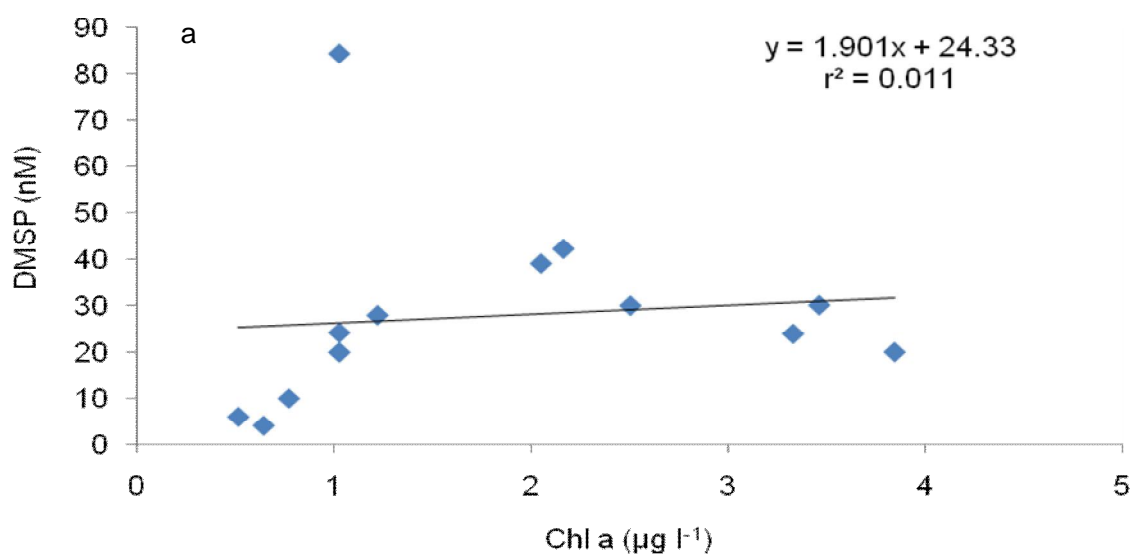


Fig. 7 Scatter plot of a) Chl a vs DMSP, b) Chl a vs DMS measured during the diurnal study

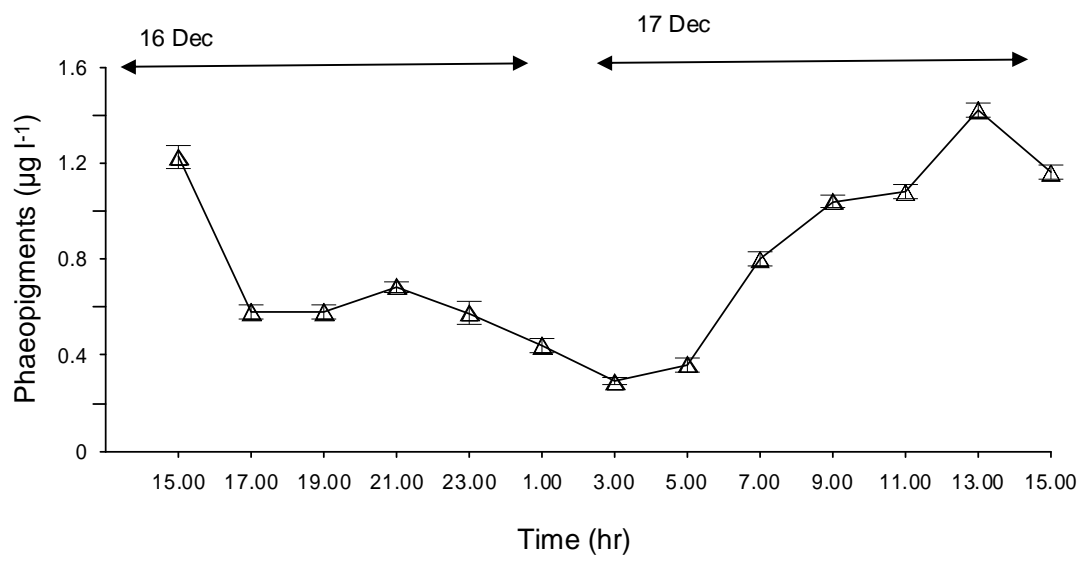


Fig. 8 Diurnal variability of Phaeopigments at Dona Paula bay

Time	Chl a ($\mu\text{g l}^{-1}$)	Phaeopigments ($\mu\text{g l}^{-1}$)
Day	2.62 \pm 0.98*	1.04 \pm 0.27
Night	0.86 \pm 0.27	0.49 \pm 0.15

Table. 4a Mean variability of Chl a, Phaeopigments, at Dona Paula bay. * (\pm SD)

Variables	Spearman r	p value
Chl a & Phaeopigments	0.917	< 0.001
Phaeopigments & pH	0.631	< 0.05
Phaeopigments & DMSP	0.674	< 0.01

Table. 4b Factors affecting Chl a and Phaeopigments during diurnal study

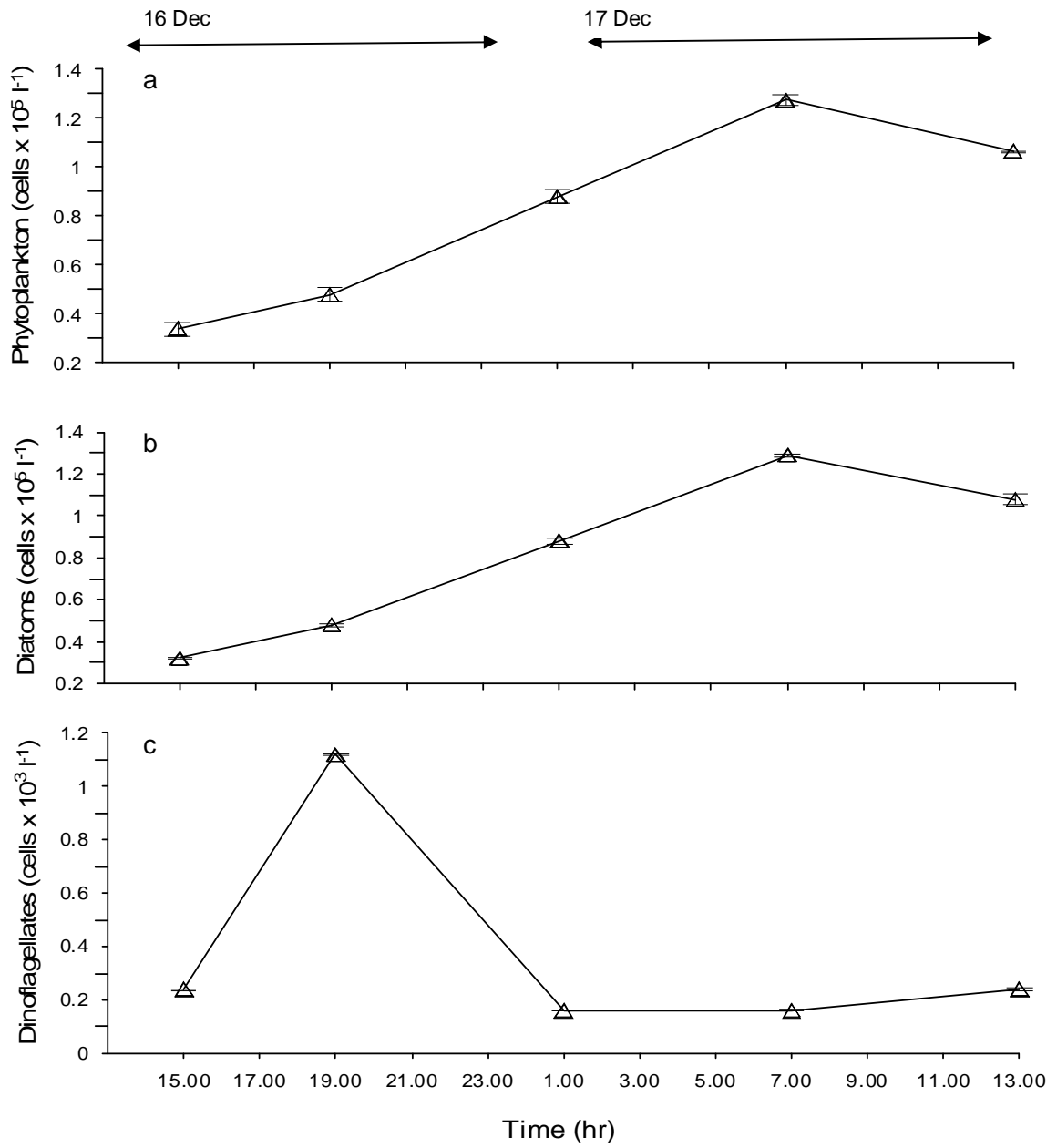


Fig. 9 Diurnal variations of a) Phytoplankton, b) Diatoms and c) Dinoflagellates at Dona Paula bay

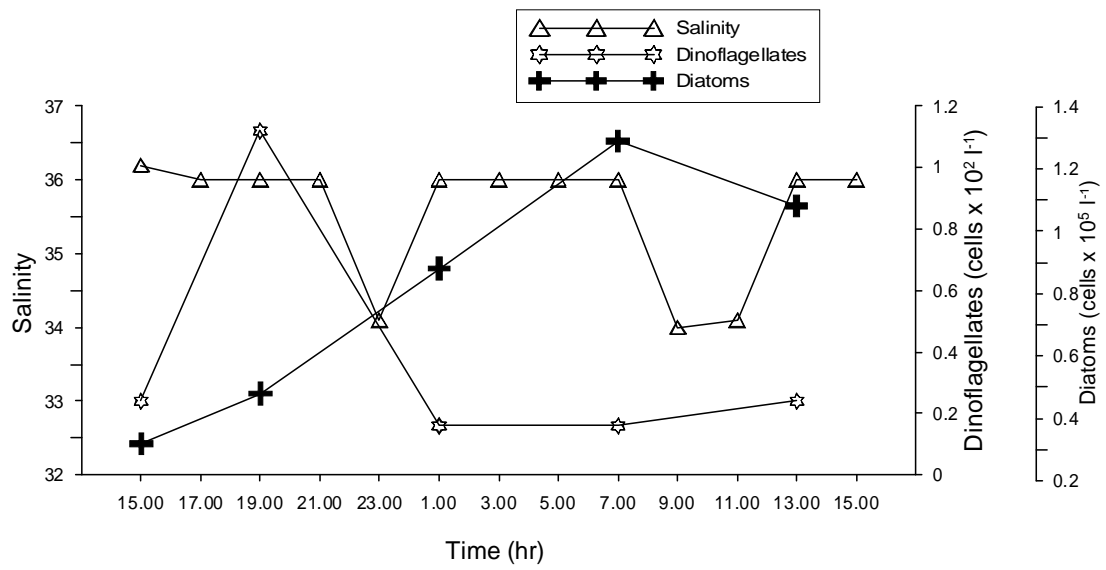


Fig. 10 Variation of Dinoflagellates and Diatoms with Salinity during diurnal study

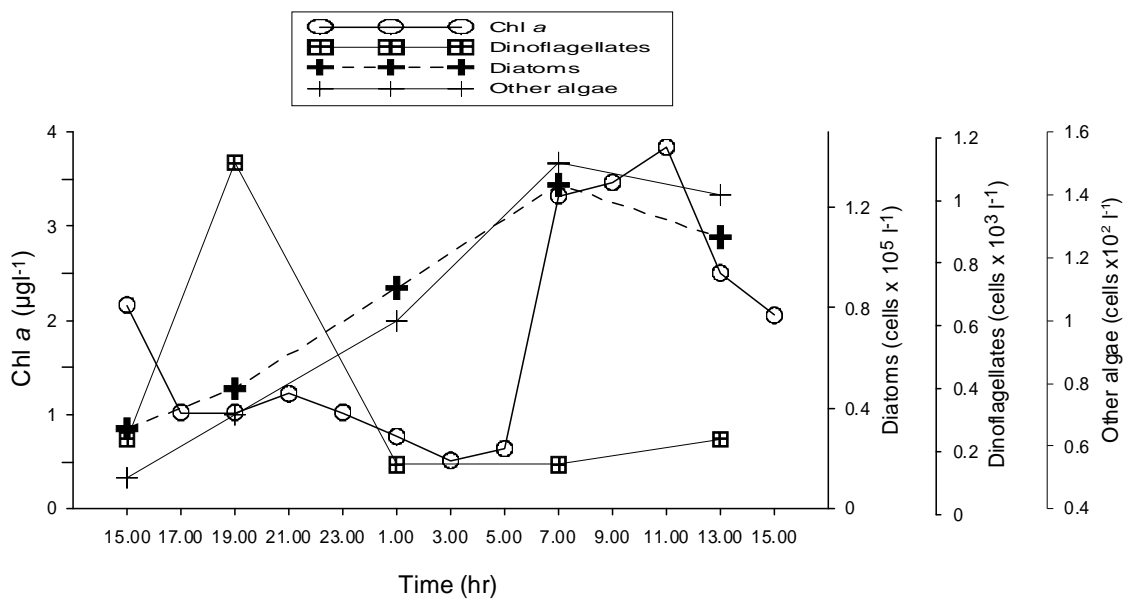


Fig. 11 Trend in Diatoms, Dinoflagellates and other algae with Chl a during diurnal study

Time	Phytoplankton (cells x10 ⁵ l ⁻¹)	Diatoms (cells x10 ⁵ l ⁻¹)	Dinoflagellates (cells x 10 ³ l ⁻¹)
Day	0.90 ± 0.49	1.08 ± 0.20	0.21 ± 0.04
Night	0.68 ± 0.28	0.41 ± 0.09	0.64 ± 0.67

Table. 5 Mean variability of Phytoplankton, Diatoms and Dinoflagellates at

Dona Paula bay * (± SD)

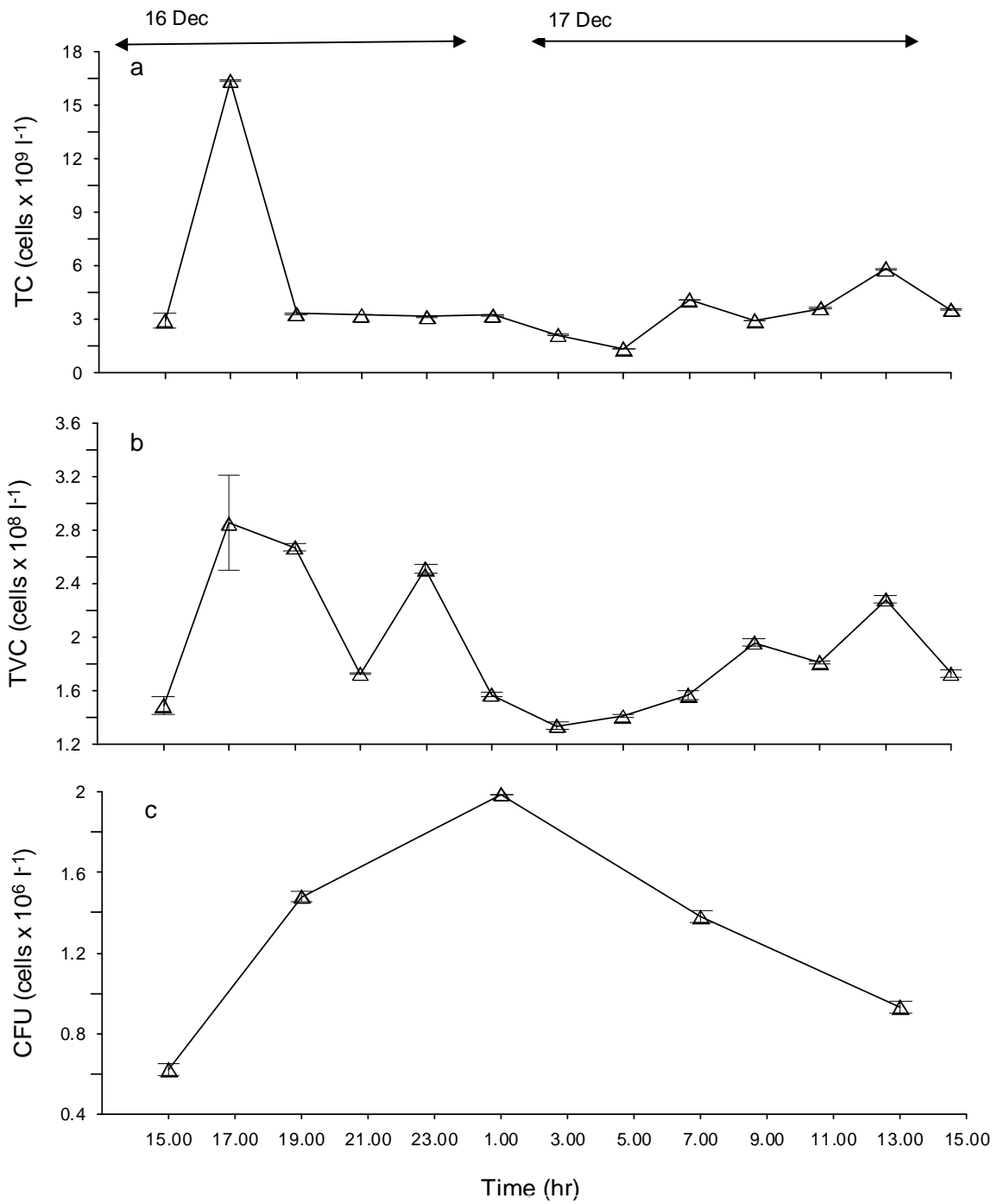


Fig. 12 Diurnal variations of a) Total Count (TC), b) Total Viable Counts (TVC) and c) Colony Forming Units (CFU) at Dona Paula bay

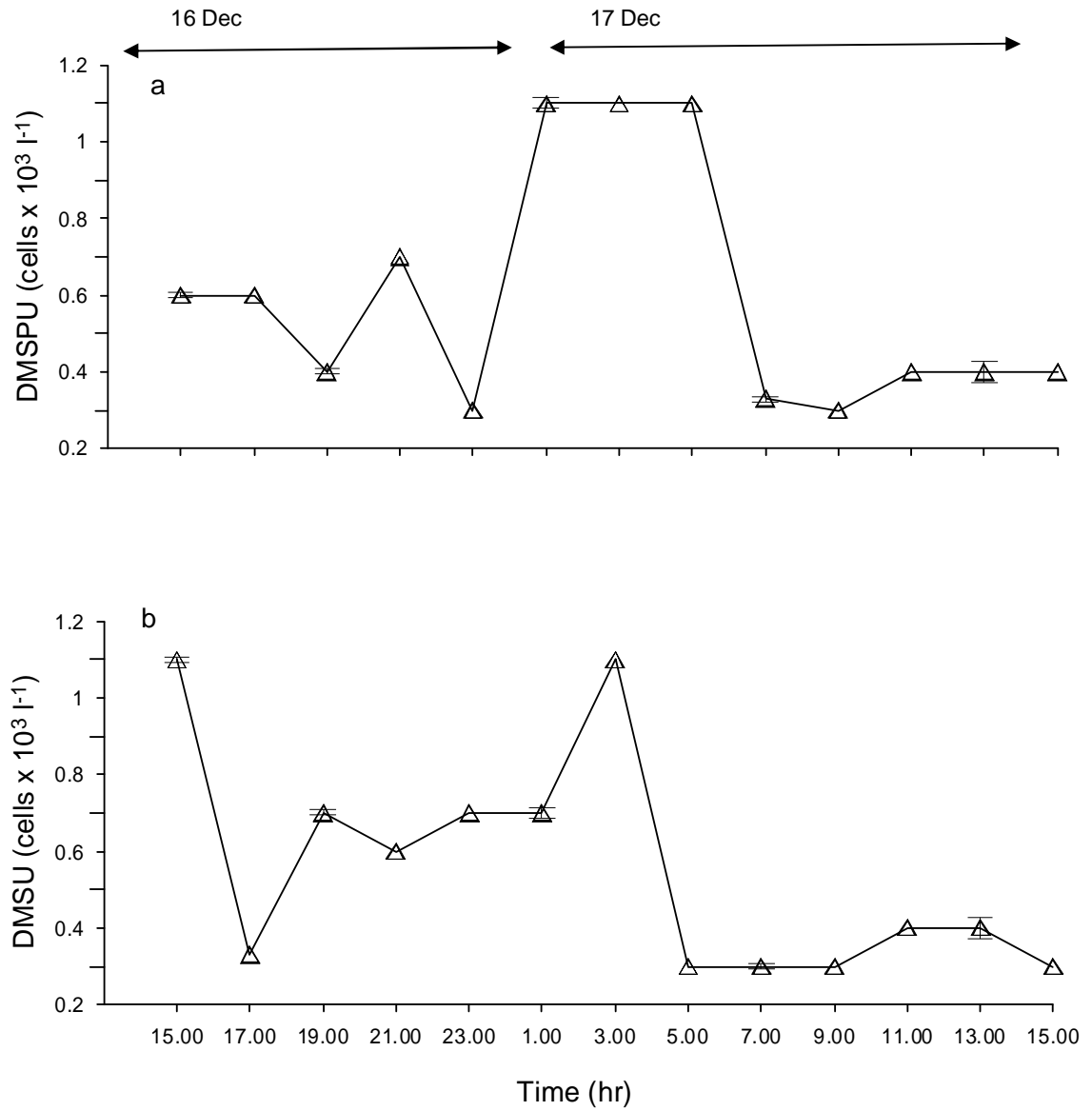


Fig. 13 Diurnal variations of DMSP Utilizers (DMSPU) and DMS Utilizers (DMSU) at Dona Paula bay

Time	TC (cells x 10 ⁹ l ⁻¹)	TVC (cells x 10 ⁸ l ⁻¹)	CFU (cells x 10 ⁶ l ⁻¹)	DMSPU (cells x10 ³ l ⁻¹)	DMSU (cells x10 ³ l ⁻¹)
Day	5.60 ± 4.84*	1.95 ± 0.47	0.97 ± 0.38	0.43 ± 0.12	0.45 ± 0.29
Night	2.72 ± 0.81	1.87 ± 0.58	1.74 ± 0.36	0.78 ± 0.37	0.68 ± 0.26

Table. 6 Mean variability of Total Counts (TC), Total Viable Counts (TVC), Colony Forming Units (CFU),

DMSP utilizers (DMSU) and DMS utilizers (DMSU) at Dona Paula bay * (± SD)

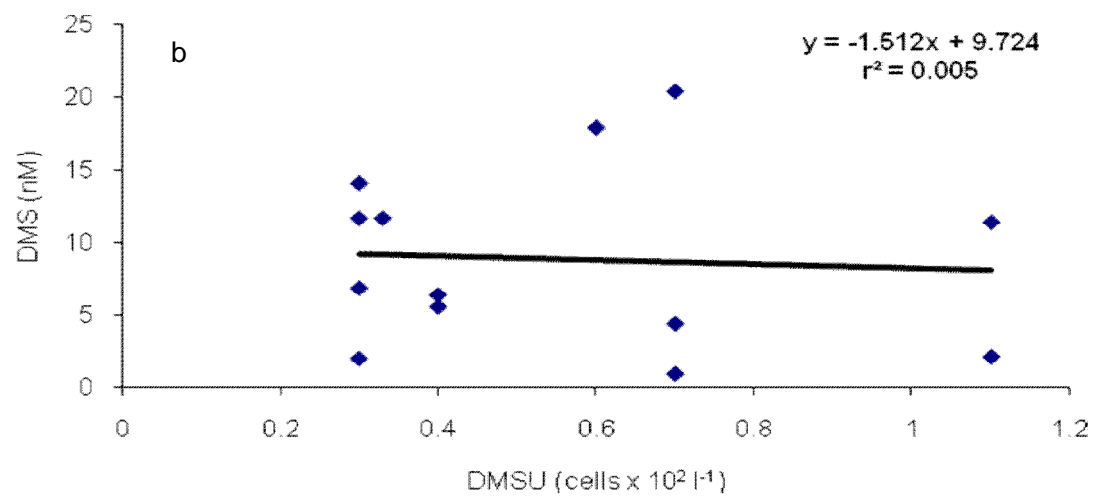
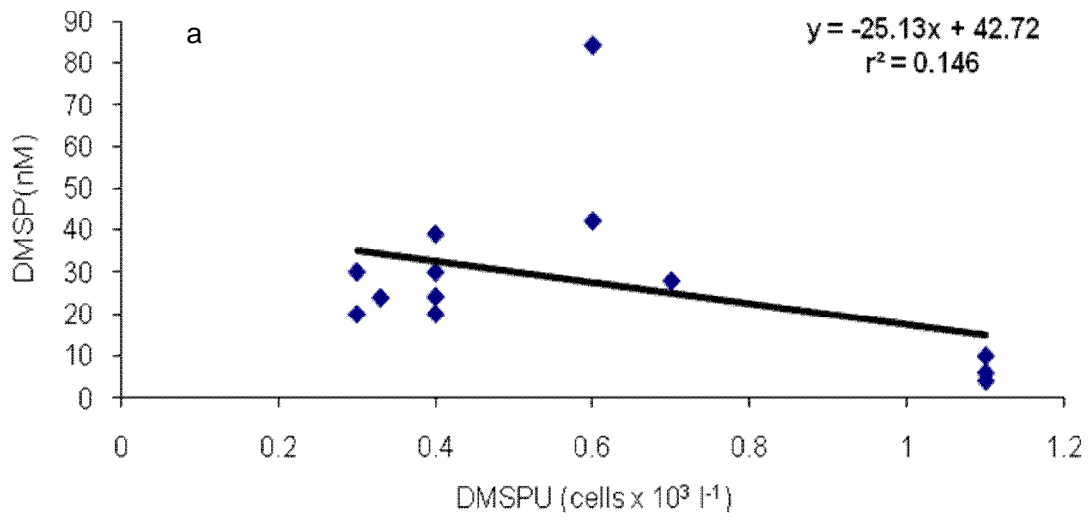


Fig. 14 Scatter plot showing diurnal variation of a) DMSPU versus DMSP, b) DMSU versus DMS

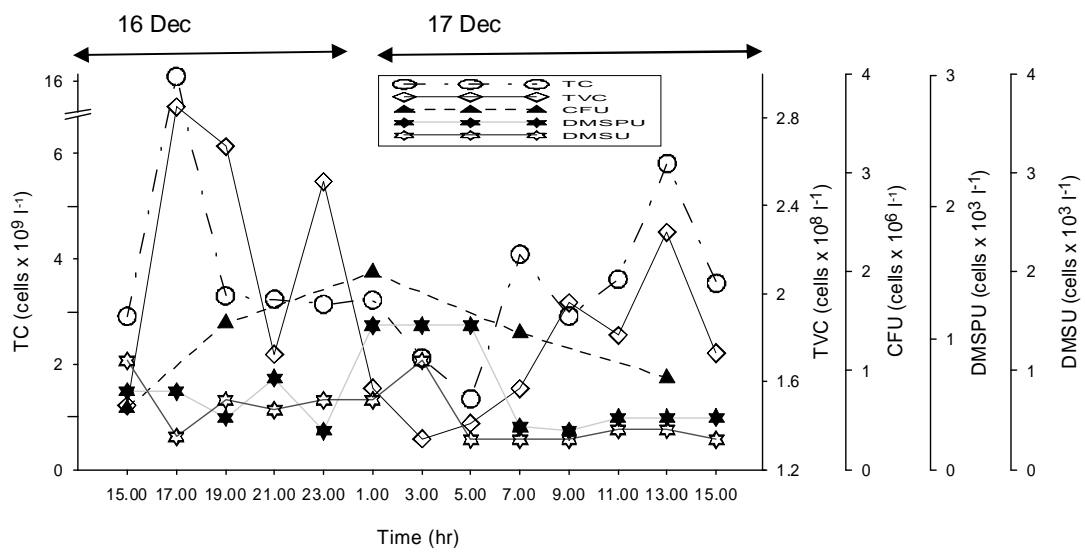


Fig. 15 Variation of DMSPU and DMSU with TC, TVC, CFU during diurnal study

Variables	Spearman r	p value
DMSPU & Chl a	-0.639	< 0.01
DMSU & Phytoplankton	-0.975	< 0.001
DMSU & Diatoms	-0.975	< 0.001
DMSPU & TVC	-0.573	< 0.05

Table. 7 Spearman correlation coefficients of Biological variables
during diurnal study at Dona Paula bay

PCA

Principal Component Analysis

Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Variation
1	1.41	51.3	51.3
2	0.401	14.6	65.8
3	0.35	12.7	78.5

Eigenvectors (Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3	
Tide		-0.075	0.042	-0.006
Temperature		0.011	0.017	0.022
Salinity		0.014	-0.009	0.006
pH		0.007	-0.019	0.004
DO		-0.083	-0.111	-0.129
Chl a		0.199	-0.257	-0.293
Phaeopigment		0.110	-0.081	-0.053
TOC		-0.112	0.691	-0.471
DMS		0.543	0.316	-0.249
DMSP		0.614	0.174	0.197
TC		0.349	-0.011	0.346
TVC		0.138	-0.095	0.142
DMSPU		-0.306	0.350	0.193
DMSU		-0.128	0.235	0.567
Wind		-0.020	-0.341	-0.270
Flux		-0.002	-0.042	-0.030

Table. 8: Eigen values and vectors of all variables during the diurnal study

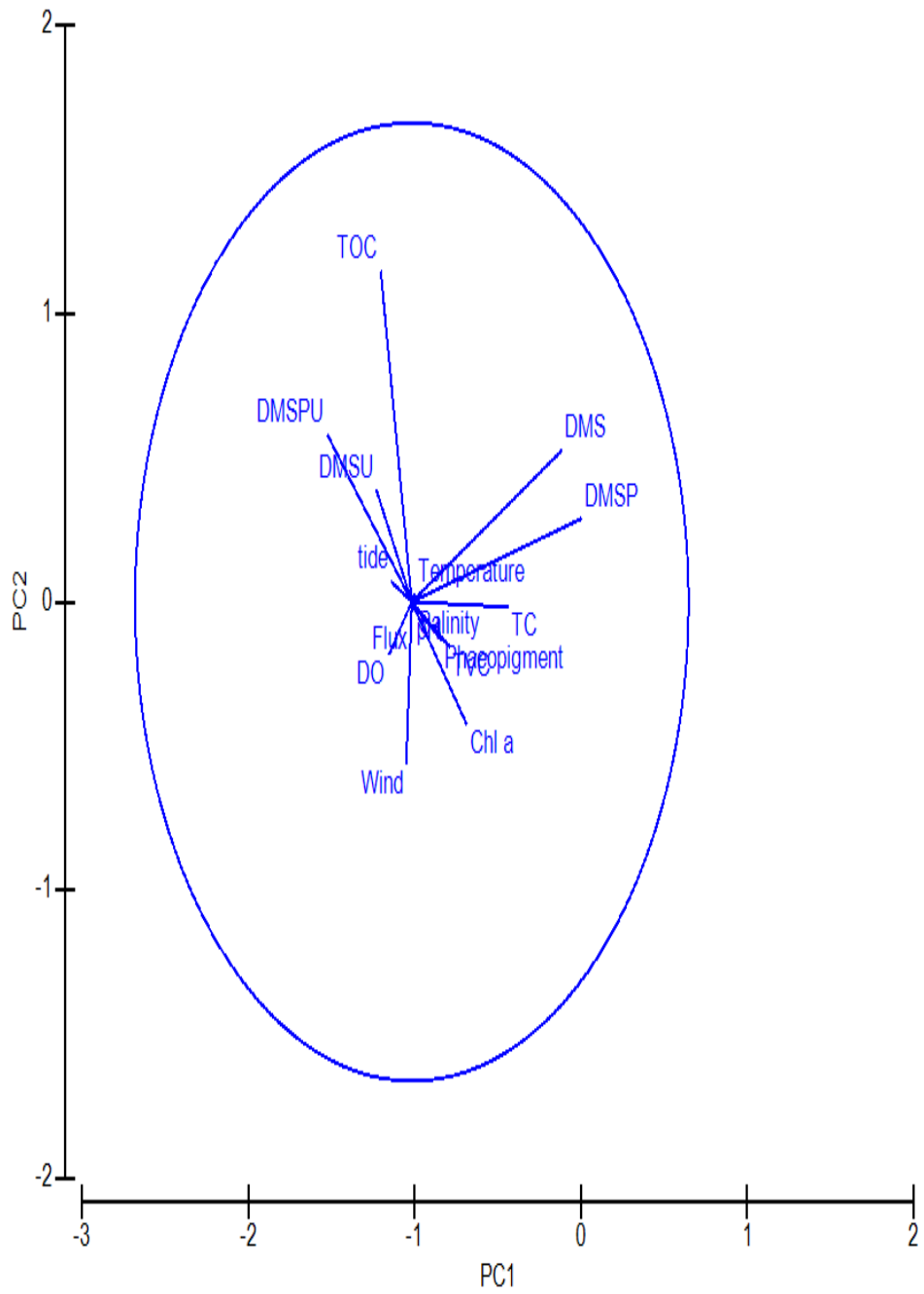


Fig. 16 The schematic representation of PCA analyses of the variables during diurnal study

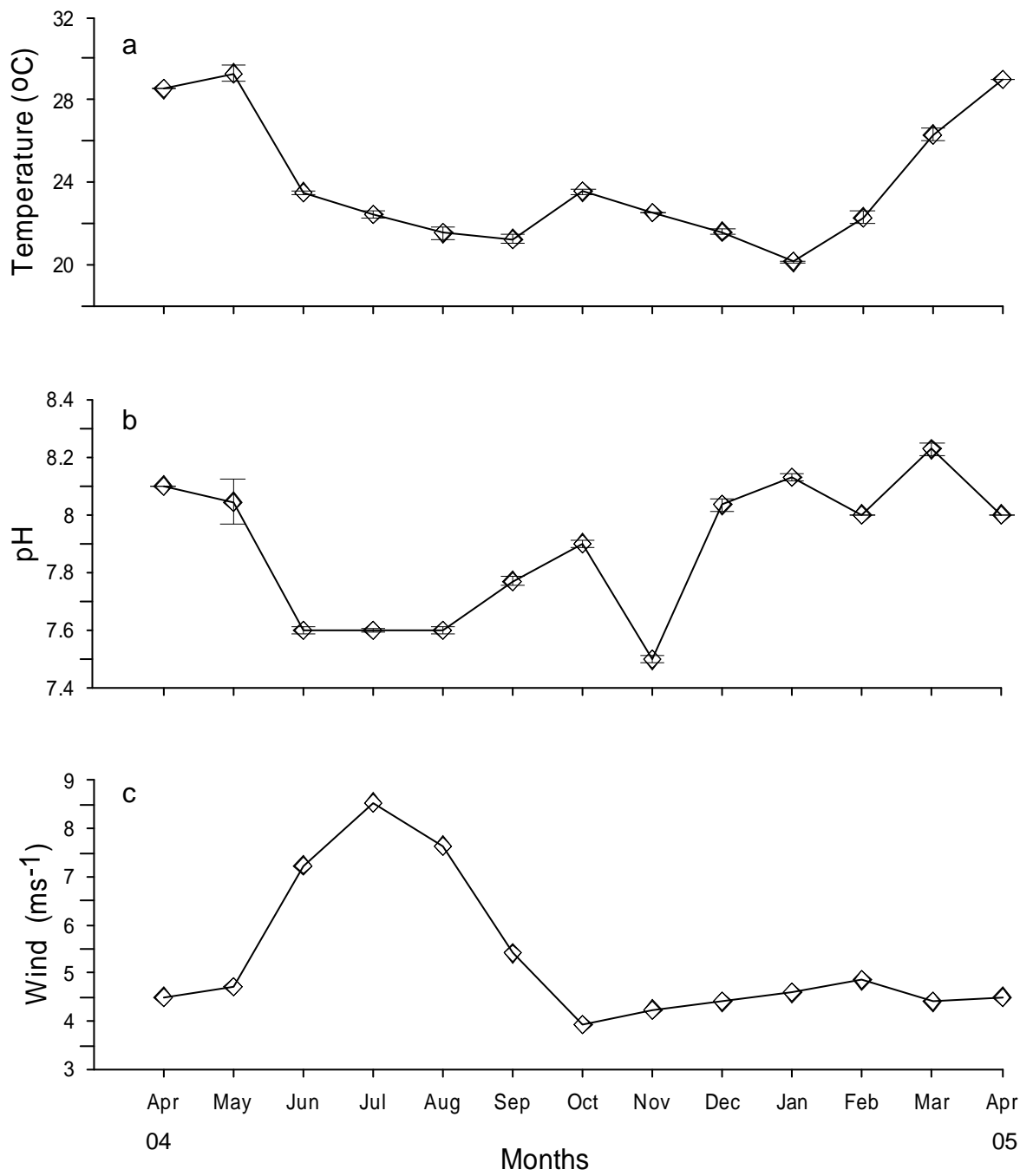


Fig. 17 Temporal variations of a) Temperature, b) pH and c) Wind at Dona Paula bay

Seasons		Temperature (°C)	pH	Wind (ms ⁻¹)
Pre-monsoon	Range	22.00 - 29.50	8.00 - 8.20	4.41 - 4.86
	Mean ± SD	27.10 ± 3.07	8.06 ± 0.09	4.60 ± 0.19
Monsoon	Range	21.00 - 23.50	7.60 - 7.70	5.40 - 8.50
	Mean ± SD	22.25 ± 1.19	7.63 ± 0.05	7.21 ± 1.32
Post-monsoon	Range	20.00 - 23.50	7.60 - 8.10	3.93 - 4.60
	Mean ± SD	21.88 ± 1.49	7.88 ± 0.22	4.30 ± 0.28

Table. 10 Mean (± SD) seasonal variation of the Temperature, pH and Wind at Dona Paula bay

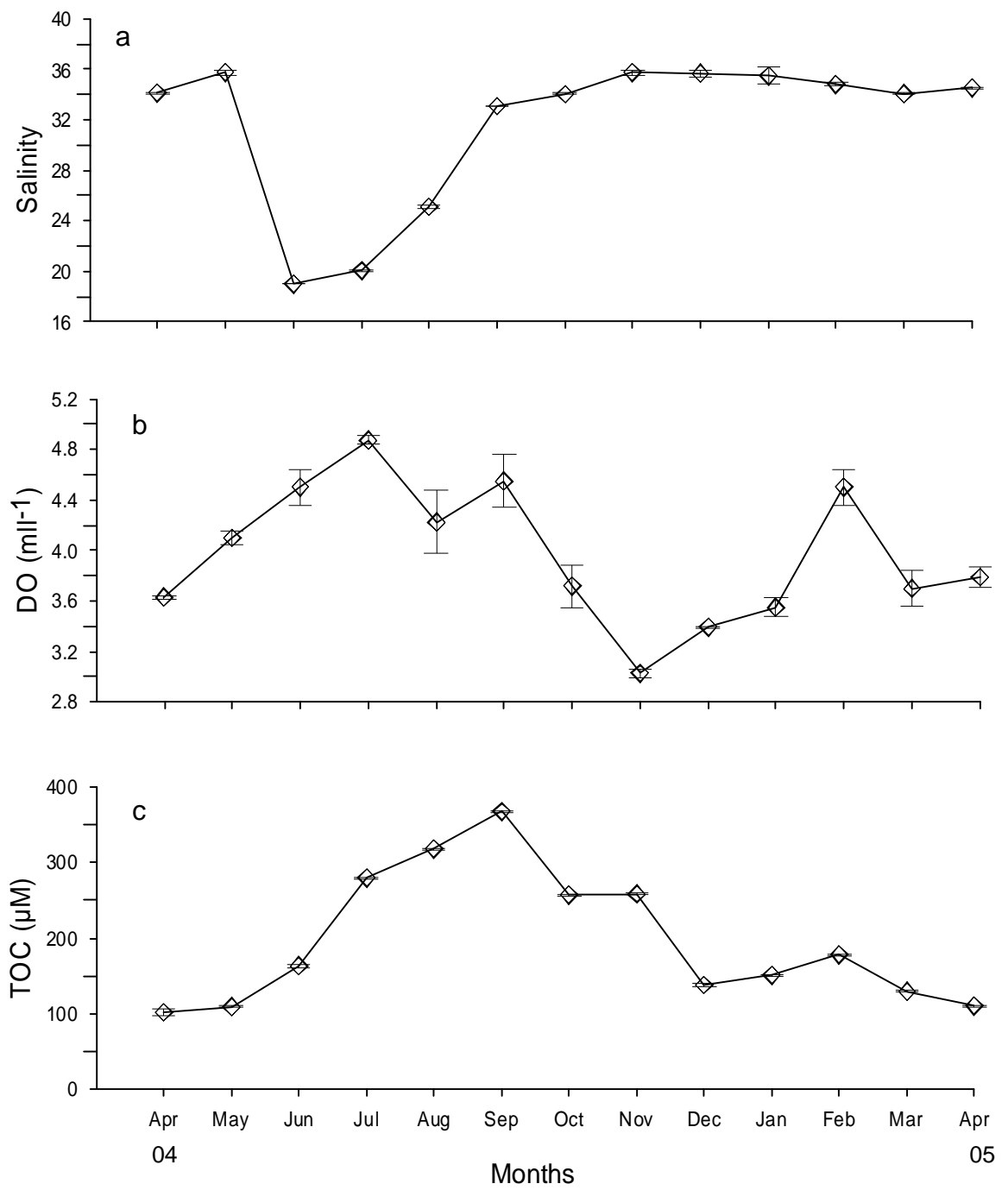


Fig. 18 Temporal variations of a) Salinity, b) Dissolved Oxygen (DO) and c) Total Organic Carbon (TOC) at Dona Paula bay

Seasons		Salinity	DO (ml l ⁻¹)	TOC (µM)
	Range	34.00 - 34.80	3.60 - 4.50	102.00 - 177.58
Pre-monsoon	Mean ± SD*	34.63 ± 0.69	3.94 ± 0.36	125.92 ± 30.66
	Range	19.00 - 33.00	4.00 - 4.90	163.12 - 366.58
Monsoon	Mean ± SD	24.25 ± 6.40	4.48 ± 0.37	281.65 ± 86.74
	Range	34.00 - 35.70	3.00 - 3.70	138.00 - 258.5
Post-monsoon	Mean ± SD	35.23 ± 0.82	3.4 ± 0.29	201.06 ± 65.69

Table. 11 Mean (± SD) seasonal variation of Salinity, Dissolved Oxygen (DO) and

Total Organic Carbon (TOC) at Dona Paula bay

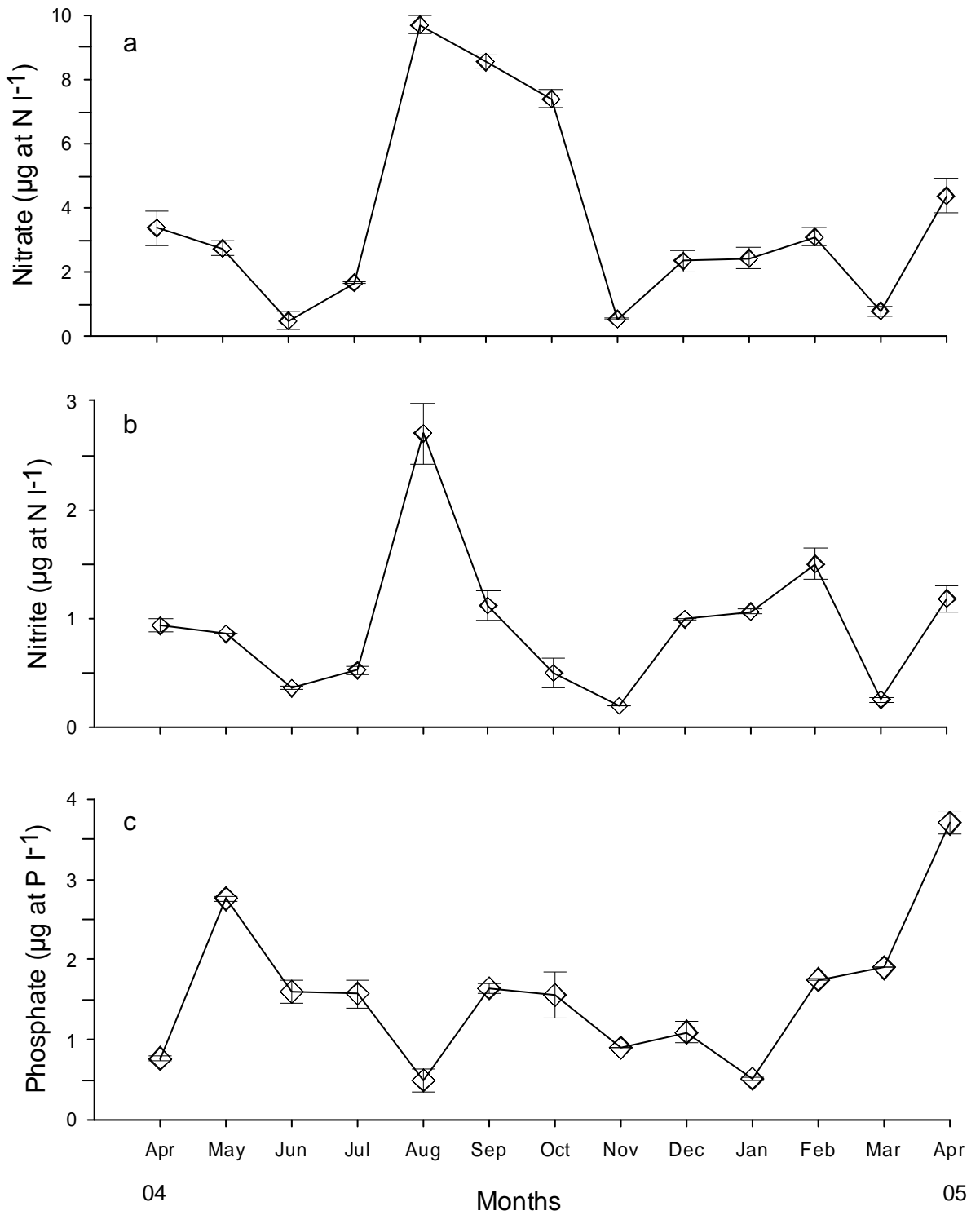


Fig. 19 Temporal variations of a) Nitrate, b) Nitrite and c) Phosphate at Dona Paula bay

Seasons		Nitrate ($\mu\text{g at N l}^{-1}$)	Nitrite ($\mu\text{g at N l}^{-1}$)	Phosphate ($\mu\text{g at P l}^{-1}$)
Pre-monsoon	Range	0.80 - 4.40	0.26 - 1.54	1.76 - 3.73
	Mean \pm SD*	2.90 \pm 1.33	1.02 \pm 0.49	2.39 \pm 0.85
Monsoon	Range	0.49 - 9.78	0.36 - 2.76	0.50 - 1.66
	Mean \pm SD	5.14 \pm 4.72	1.19 \pm 1.1	1.35 \pm 0.57
Post-monsoon	Range	0.55 - 7.39	0.20 - 1.10	0.50 - 1.57
	Mean \pm SD	3.20 \pm 2.93	0.70 \pm 0.42	1.02 \pm 0.44

Table. 12 Mean (\pm SD) seasonal variations of Nitrate, Nitrite and Phosphate at Dona Paula bay

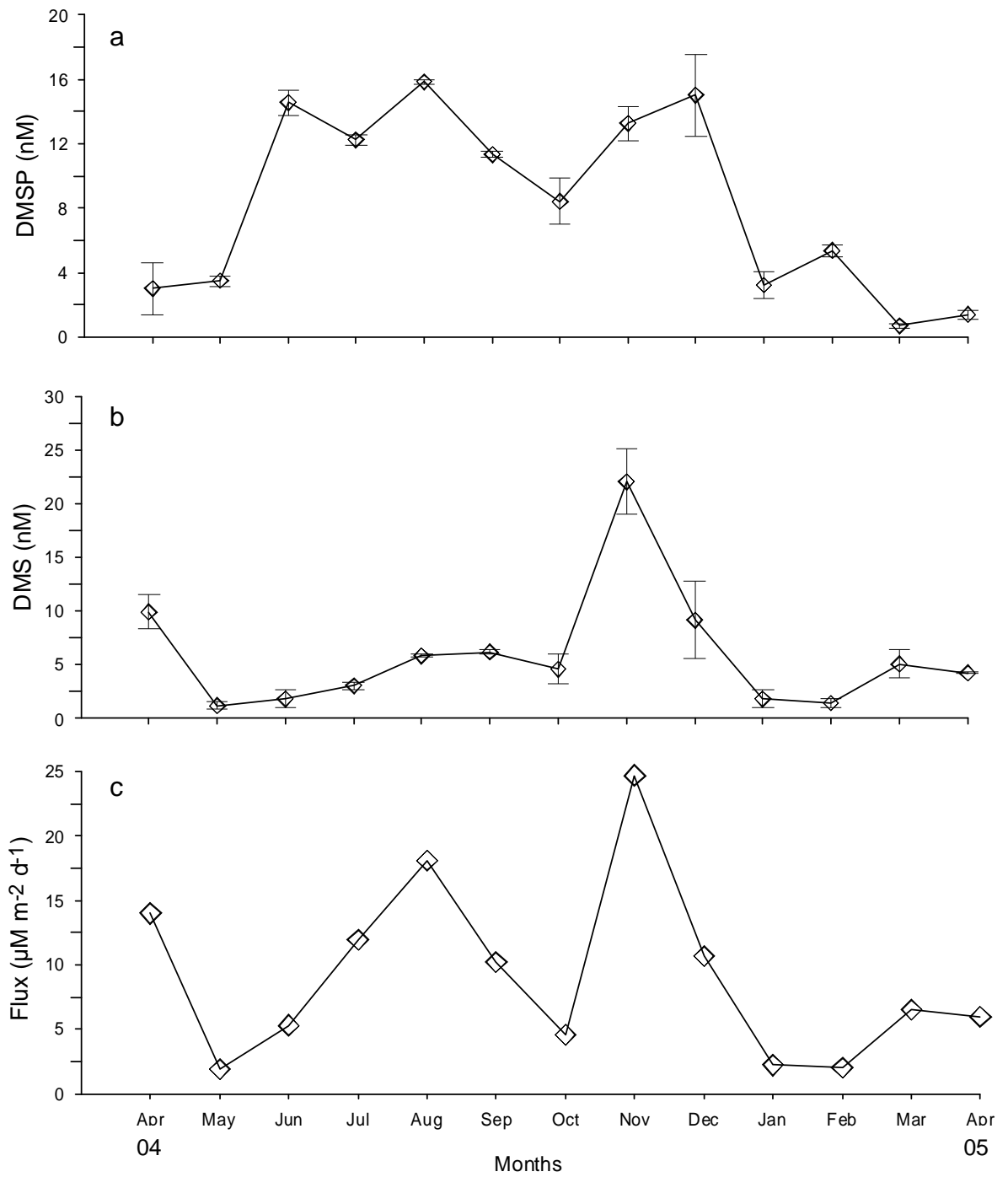


Fig. 20 Temporal variations of a) DMSP, b) DMS and c) flux at Dona Paula bay

Seasons		DMSP (nM)	DMS (nM)	Flux ($\mu\text{M m}^{-2} \text{d}^{-1}$)
Pre-monsoon	Range	0.69 - 5.35	1.20 - 9.95	1.91 - 13.99
	Mean \pm SD*	2.80 \pm 1.84	4.37 \pm 3.53	6.08 \pm 4.92
Monsoon	Range	11.20 - 15.80	1.80 - 6.20	5.32 - 18.11
	Mean \pm SD	13.40 \pm 2.09	4.20 \pm 2.14	11.4 \pm 5.28
Post-monsoon	Range	3.20 - 15.01	1.80 - 22.23	2.20 - 24.70
	Mean \pm SD	9.96 \pm 5.28	9.46 \pm 9.03	10.5 \pm 10.09

Table. 13 Mean (\pm SD) seasonal variation of DMS, DMSP and flux at Dona Paula bay

Variables	Spearman r	p value
Temperature & Phosphate	0.61	< 0.05
Temperature & TOC	-0.66	< 0.01
pH & TOC	-0.75	< 0.001
Salinity & DO	-0.64	<0.01
Salinity & Wind	-0.58	<0.05
Nitrate & Nitrite	0.73	<0.01
DMS & DO	-0.59	<0.05
DMS & Flux	0.84	<0.0001
DMSP & pH	-0.80	=0.001
DMSP & TOC	0.64	<0.01

Table.14 Spearman correlation coefficients of Physicochemical variables during annual study at Dona Paula bay

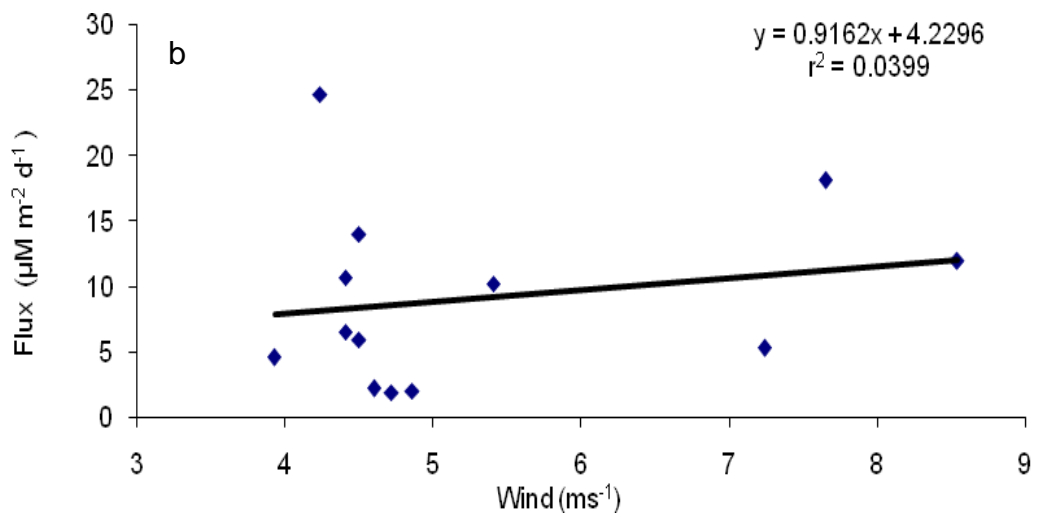
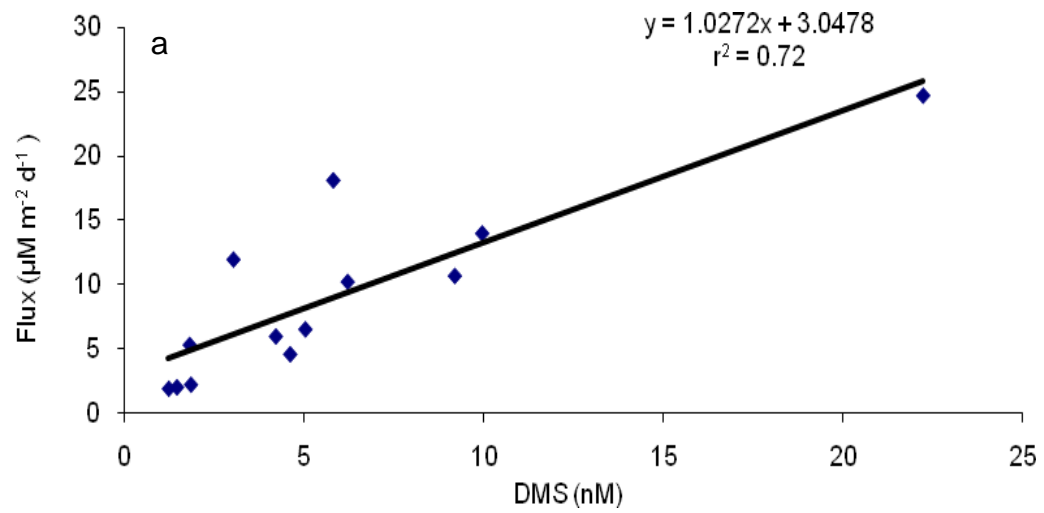


Fig. 21 Scatter plot of a) Flux versus DMS, b) Flux versus wind

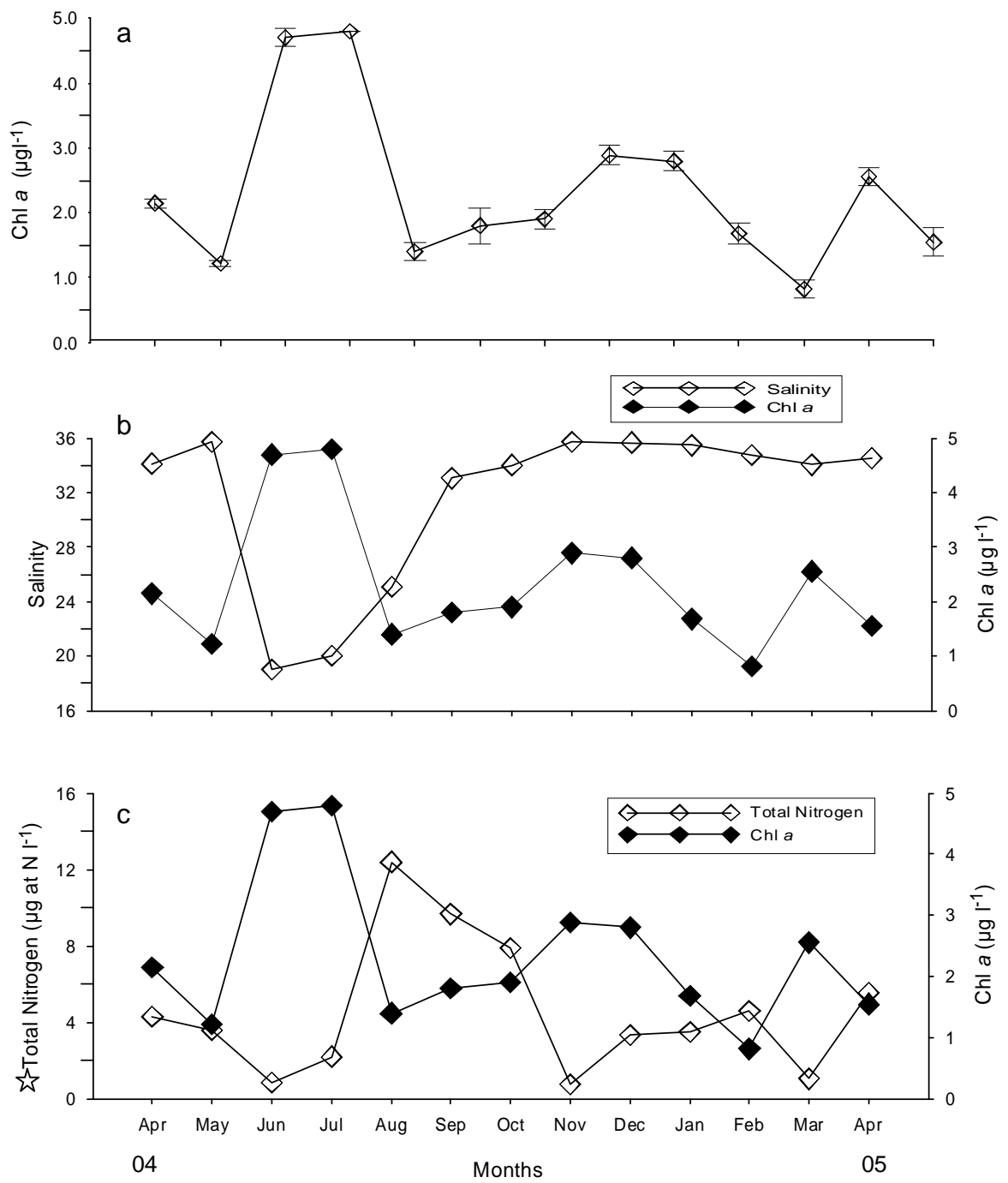


Fig. 22 Temporal variations of a) Chlorophyll a (Chl a), b) Chl a with Salinity c) Chl a with Total Nitrogen (\star Nitrate + Nitrite) at Dona Paula bay

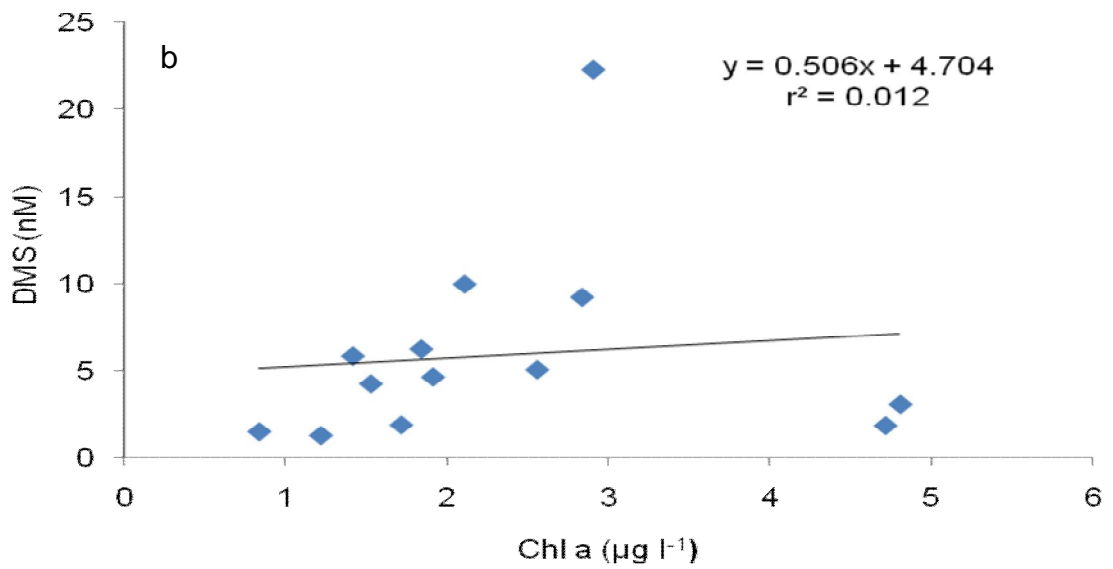
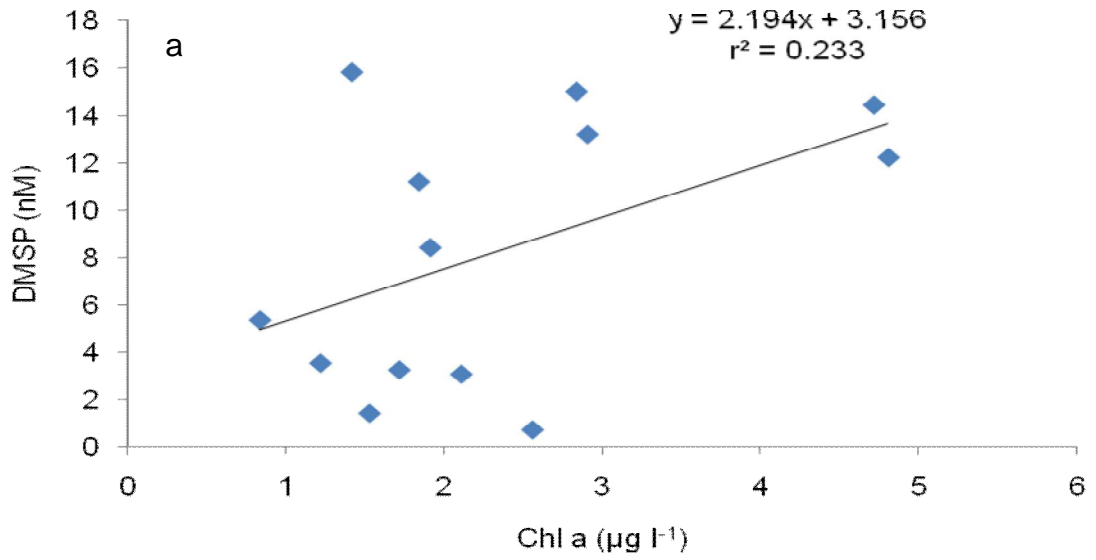


Fig. 23 Scatter plot of a) DMSP vs Chl a, b) DMS vs Chl a

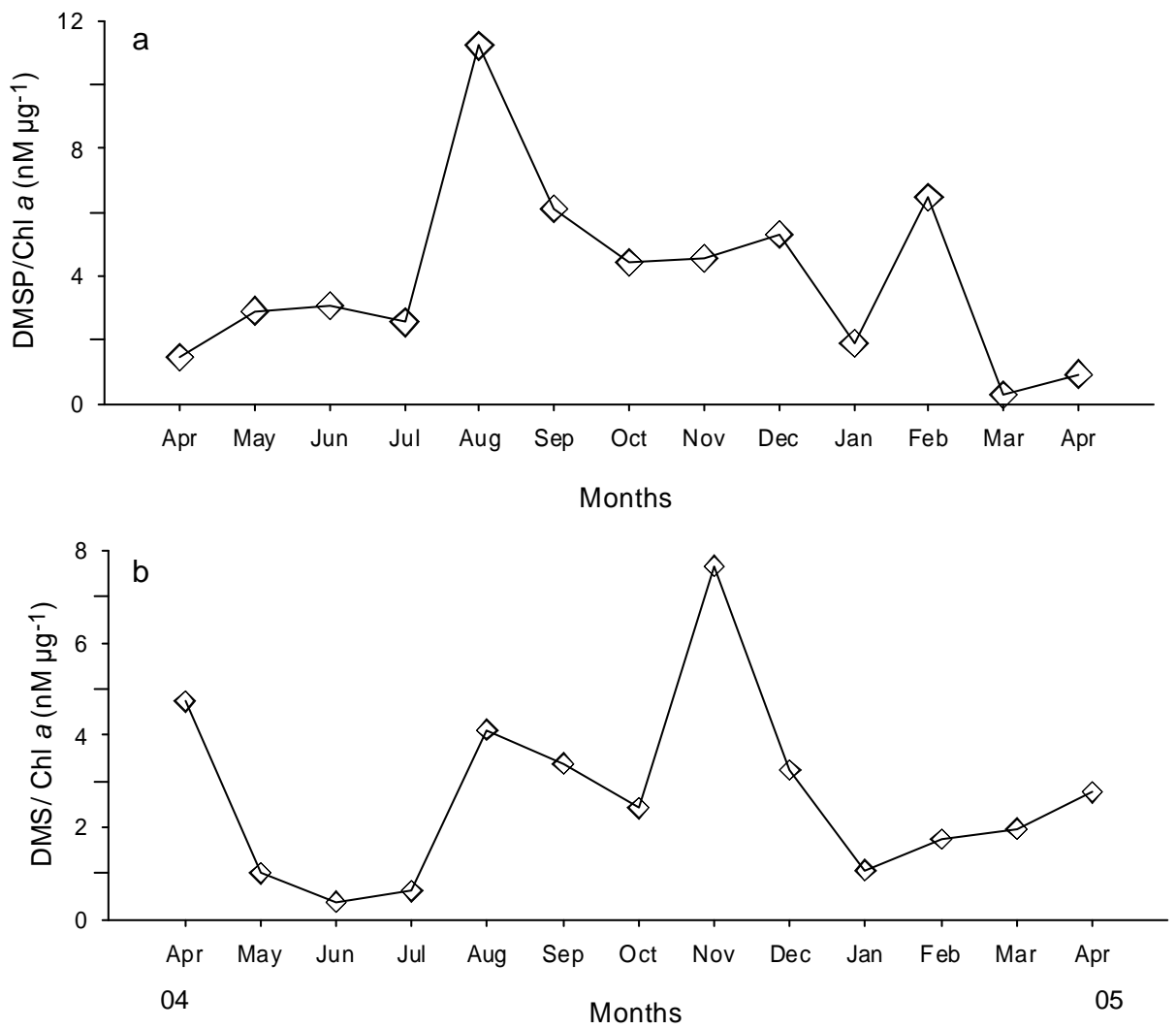


Fig. 24 Temporal distribution of a) DMSP/Chl a and b) DMS/Chl a at Dona Paula bay

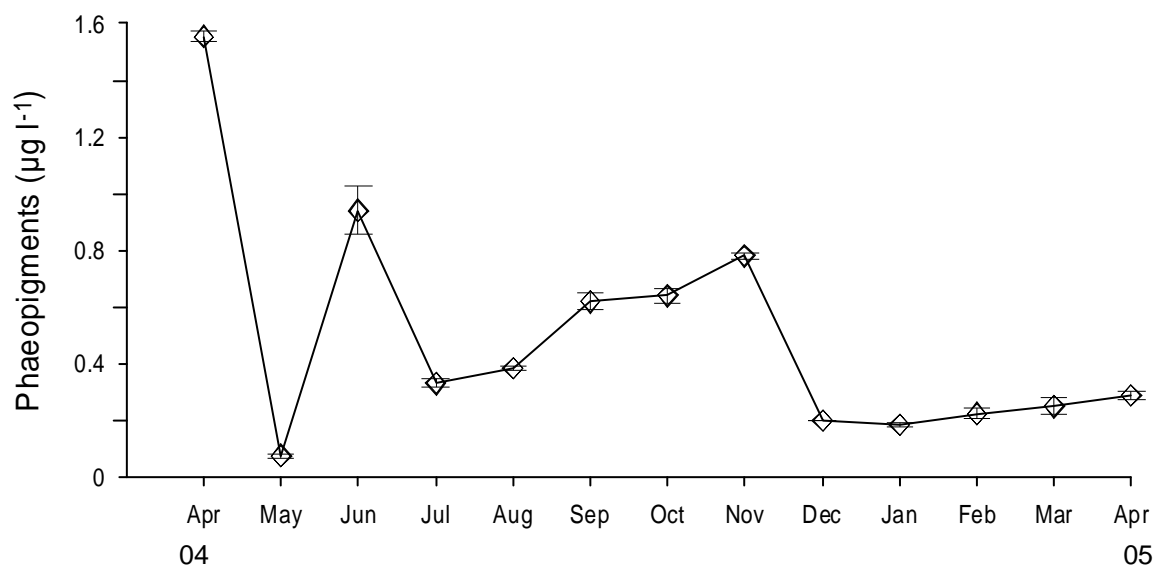


Fig. 25 Temporal variations of phaeopigments at Dona Paul bay

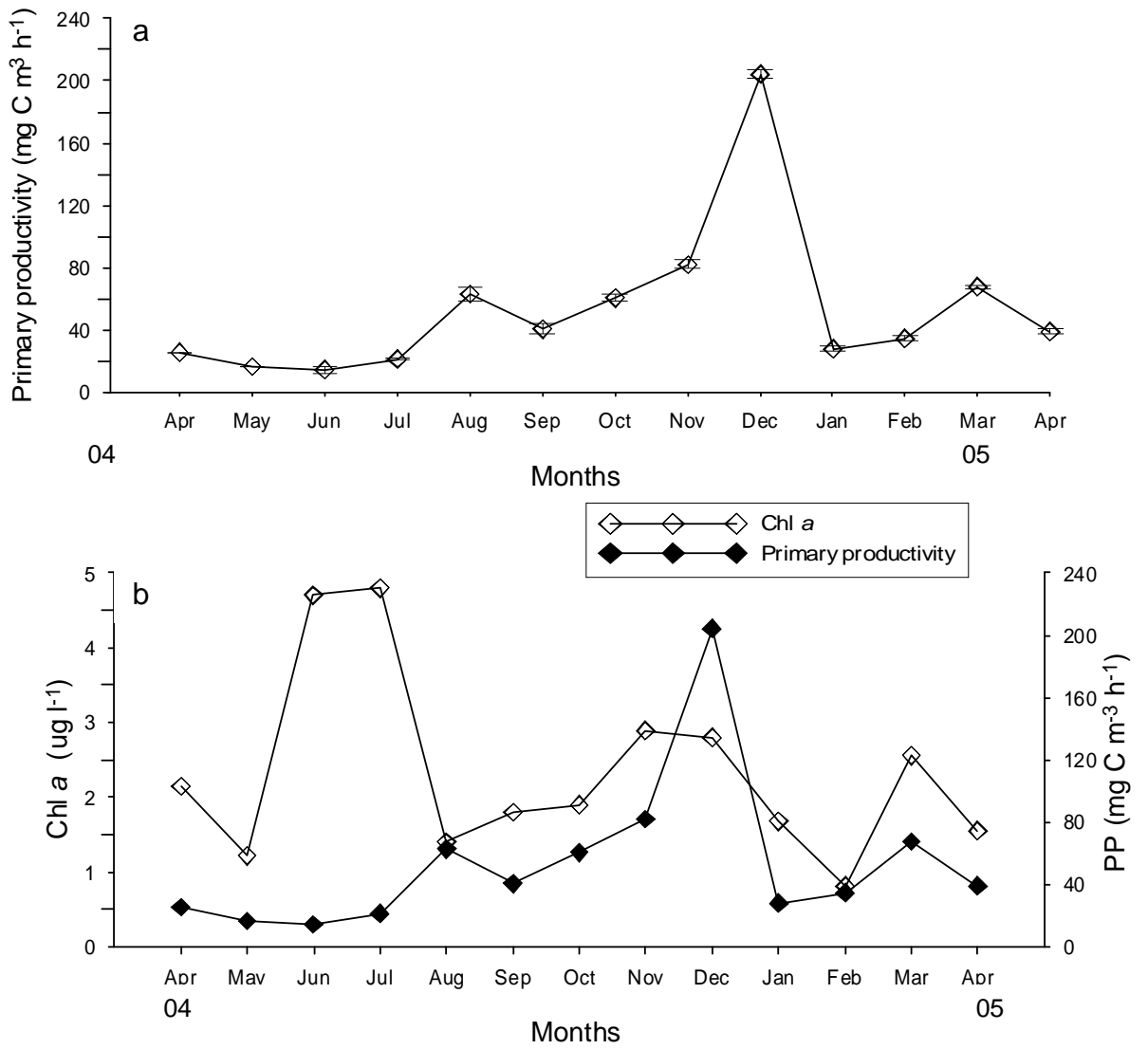


Fig. 26 Temporal variations of a) Primary productivity b) PP and Chl a at Dona Paula bay.

Seasons		Chl a ($\mu\text{g l}^{-1}$)	Phaeopigments ($\mu\text{g l}^{-1}$)	Primary Productivity ($\text{mg.C m}^{-3} \text{h}^{-1}$)
Pre-monsoon	Range	1.10 - 2.55	0.23 - 1.56	16.64 - 67.69
	Mean \pm SD*	1.58 \pm 0.57	0.62 \pm 0.57	36.63 \pm 19.42
Monsoon	Range	1.41 - 4.80	0.33 - 0.94	11.52 - 63.00
	Mean \pm SD	3.19 \pm 1.82	0.57 \pm 0.28	32.26 \pm 24.23
Post-monsoon	Range	1.71 - 2.90	0.19 - 0.79	28.05 - 204.37
	Mean \pm SD	2.33 \pm 0.62	0.46 \pm 0.3	93.80 \pm 77.01

Table 15: Mean (\pm SD) seasonal variation of Chl a, Phaeopigments and Primary Productivity at

Dona Paula bay

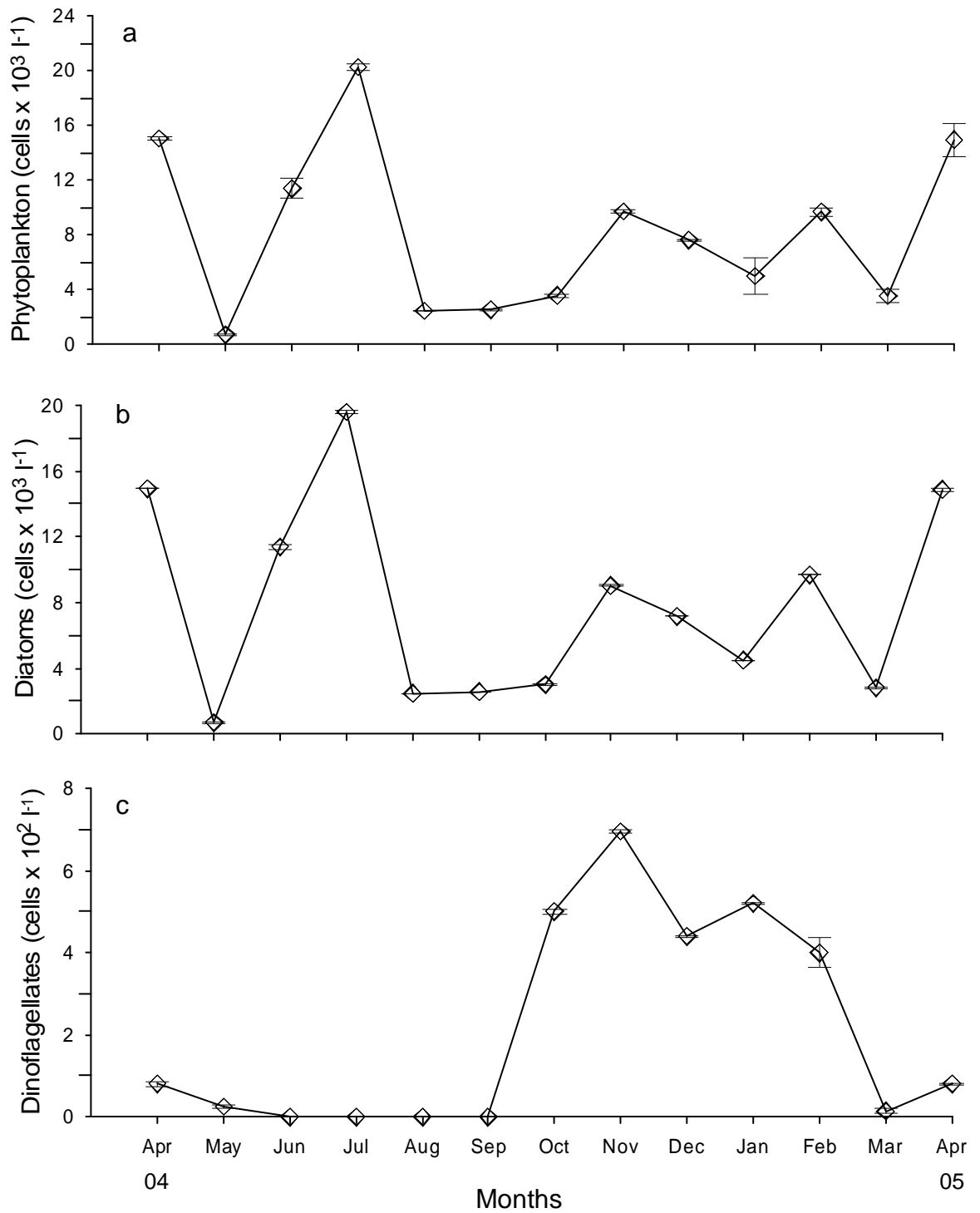


Fig. 27 Temporal variations of a) Phytoplanktons, b) Diatoms, and c) Dinoflagellates at Dona Paula bay

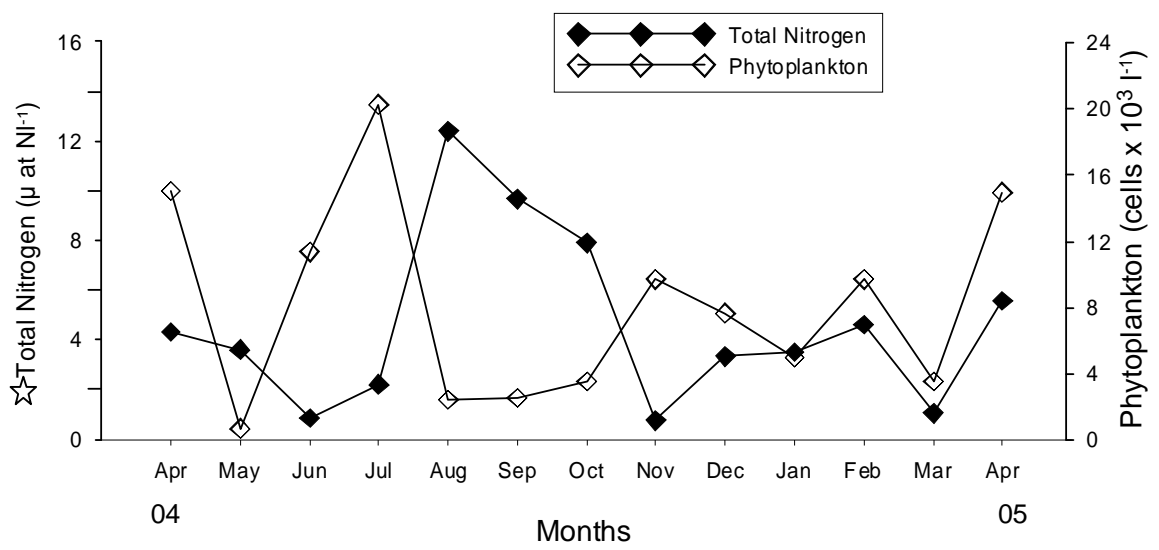


Fig. 28 Variation in Phytoplankton and Total Nitrogen (\star Nitrate + Nitrite)

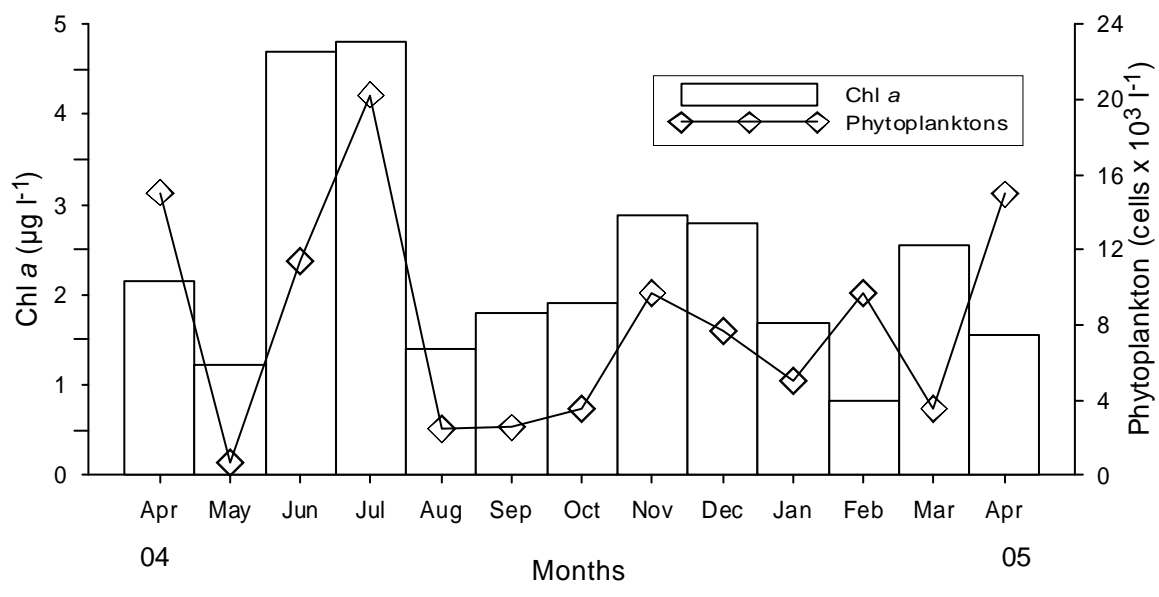


Fig. 29 Trend in Phytoplankton density and Chl a at Dona Paula bay

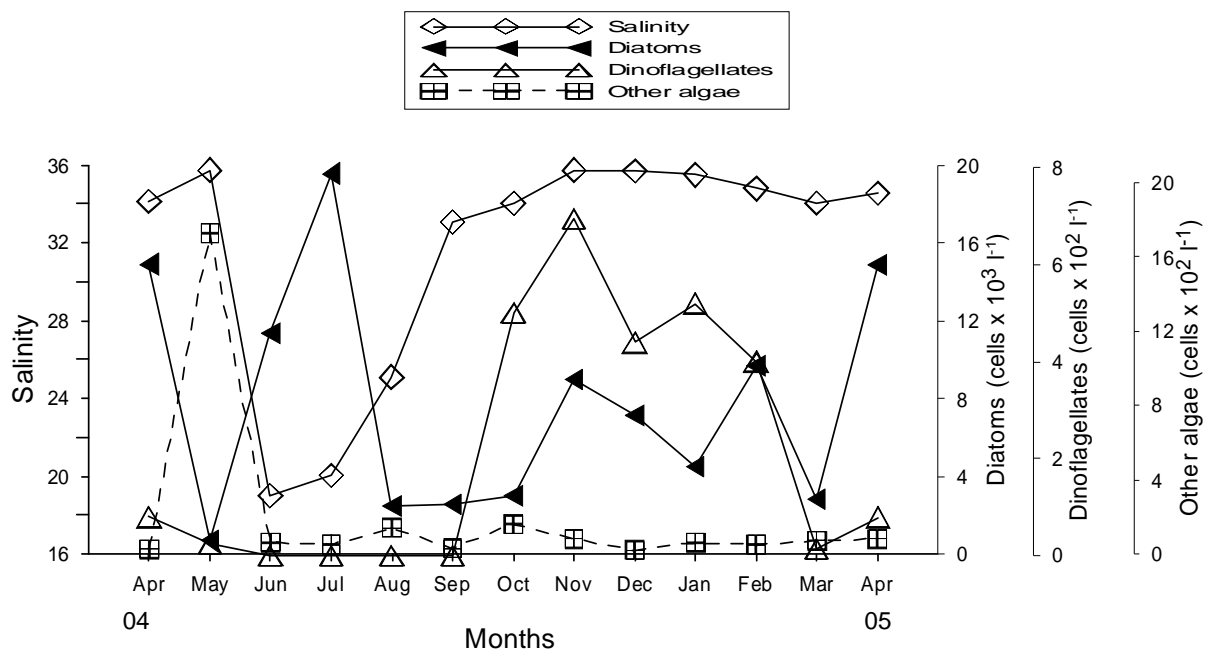


Fig. 30 Variation algal species with salinity at Dona Paula bay

Seasons		Phytoplankton (cells x 10 ³ l ⁻¹)	Diatoms (cells x 10 ³ l ⁻¹)	Dinoflagellates (cells x 10 ² l ⁻¹)
Pre-monsoon	Range	0.70 – 15.00	0.68 - 14.93	0.15 – 4.00
	Mean ± SD*	8.77 ± 6.53	8.59 ± 6.65	1.2 ± 1.59
Monsoon	Range	2.41 - 20.24	2.40 - 19.60	0.00
	Mean ± SD	9.15 ± 8.49	8.99 ± 8.22	0.00
Post-monsoon	Range	3.50 - 9.72	3.00 - 9.03	4.4 - 6.95
	Mean ± SD	6.46 ± 2.76	5.92 ± 2.69	5.39 ± 1.1

Table. 16 Mean (±SD) seasonal variation of Phytoplankton, Diatoms and Dinoflagellates at Dona Paula bay

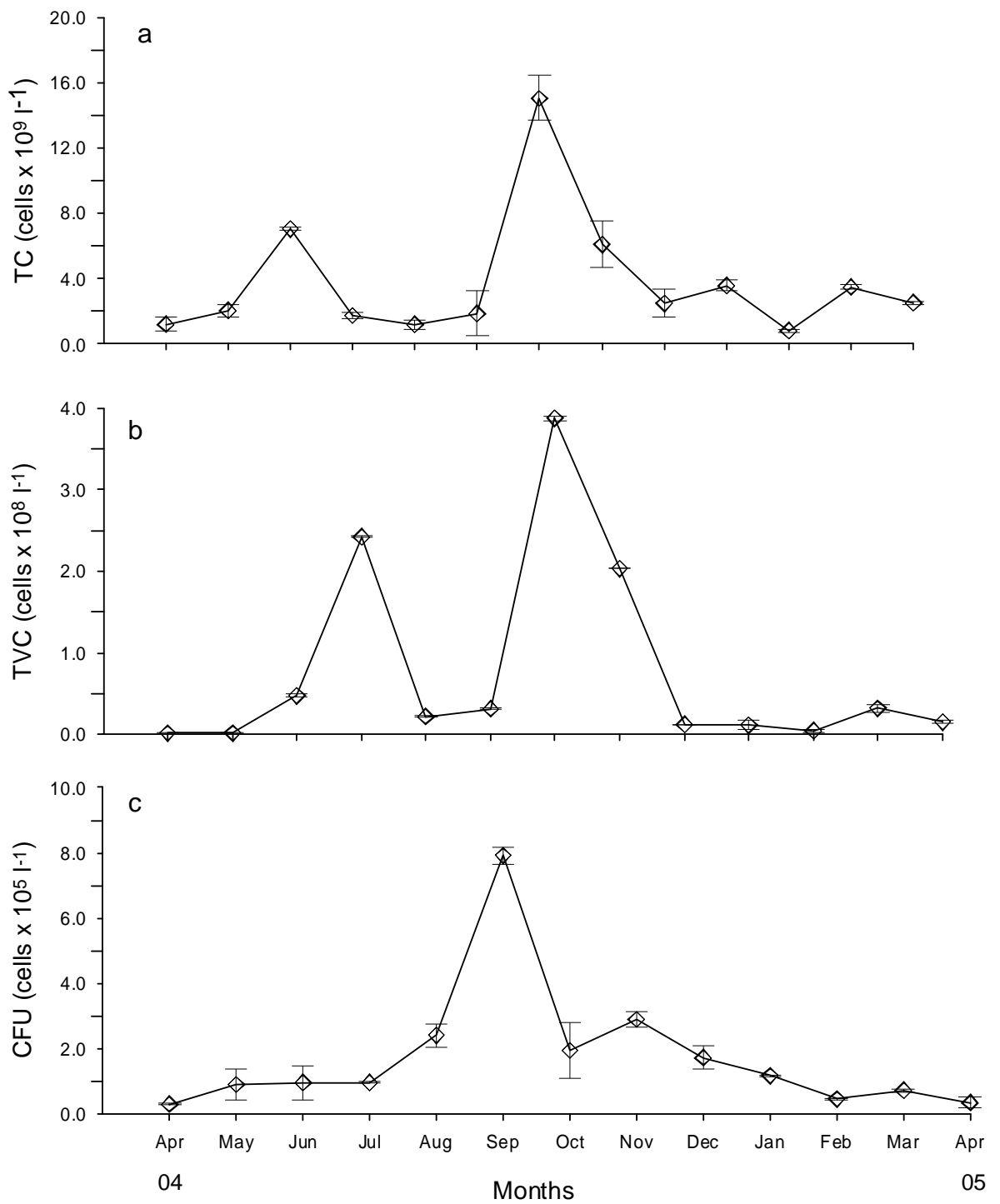


Fig. 31 Temporal variations of a) Total Counts (TC), b) Total Viable Counts (TVC), c) Colony Forming Units (CFU) at Dona Paula bay

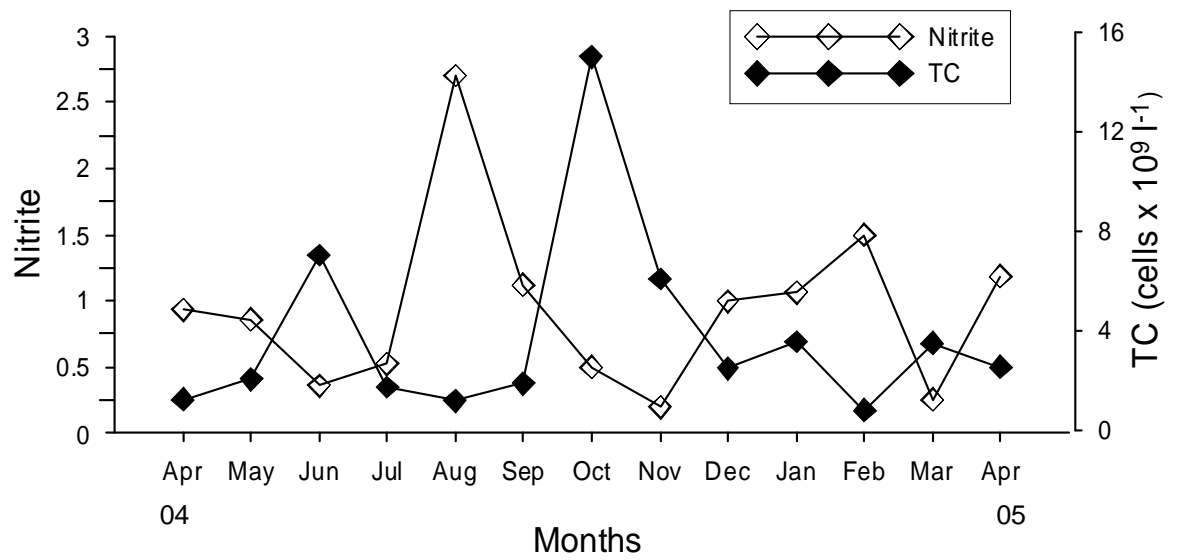


Fig. 32 Variation of TC and nitrite distribution at Dona Paula bay

Seasons		TC (cells x 10 ⁹ l ⁻¹)	TVC (cells x 10 ⁸ l ⁻¹)	CFU (cells x 10 ⁶ l ⁻¹)
Pre-monsoon	Range	0.83 - 3.50	0.03 - 0.73	0.05 - 0.73
	Mean ± SD*	2.00 ± 1.06	0.20 ± 0.30	0.24 ± 0.3
Monsoon	Range	1.18 - 7.06	0.40 - 3.15	0.97 - 7.99
	Mean ± SD	2.97 ± 2.75	2.08 ± 1.14	3.7 ± 3.34
Post-monsoon	Range	2.40 - 15.02	1.14 - 3.87	1.15 - 2.87
	Mean ± SD	6.82 ± 5.67	2.07 ± 1.27	1.93 ± 0.71

Table. 17 Mean (±SD) seasonal variation of Total Counts (TC), Total Viable Counts (TVC), Colony Forming Units (CFU) at Dona Paula bay

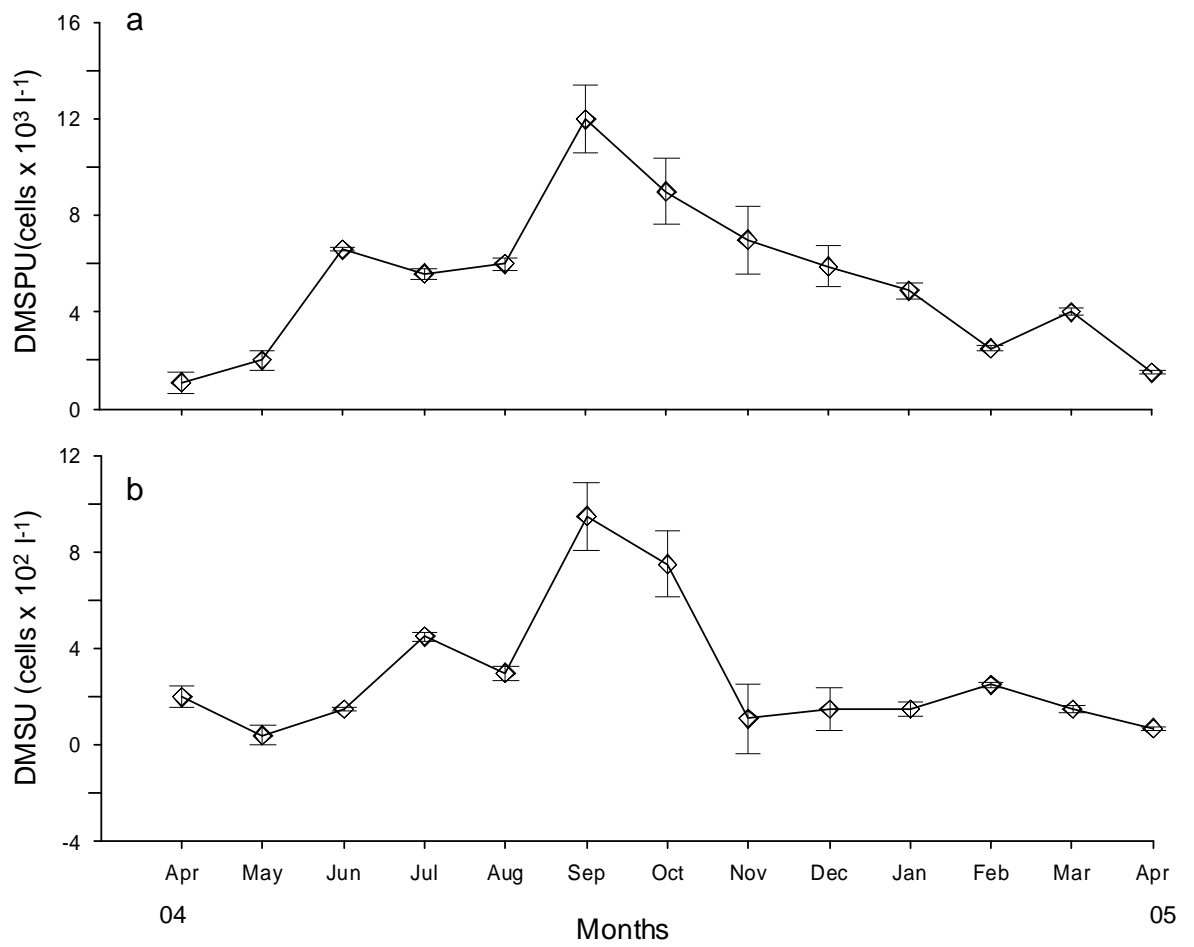


Fig. 33 Temporal variations of a) DMSPU and b) DMSU at Dona Paula bay

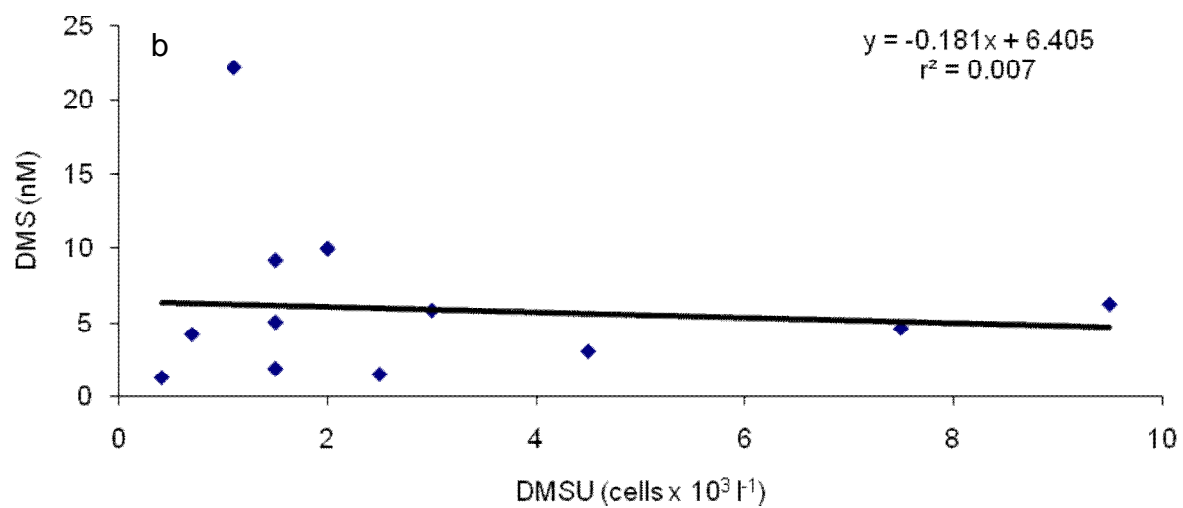
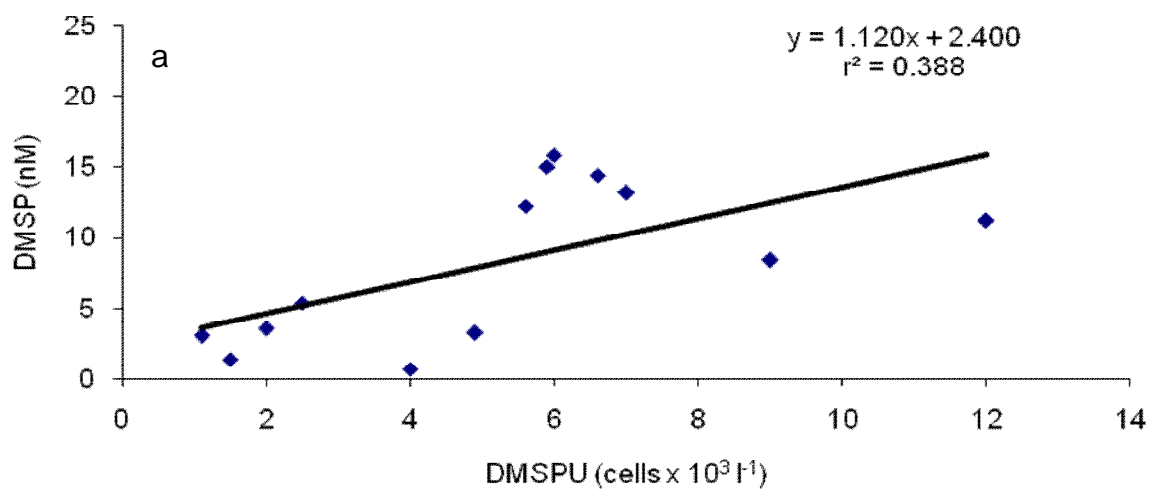


Fig. 34 Scatter plot of a) DMSP versus DMSPU, b) DMS versus DMSU

Seasons		DMSPU (cells x 10 ³ l ⁻¹)	DMSU (cells x 10 ² l ⁻¹)
Pre-monsoon	Range	1.10 - 4.00	0.41- 2.50
	Mean ± SD*	2.20 ± 1.13	1.42 ± 0.87
Monsoon	Range	5.60 -12.00	1.50 - 9.50
	Mean ± SD	7.55 ± 2.99	4.63 ± 3.47
Post-monsoon	Range	4.90 - 9.00	1.10 - 7.50
	Mean ± SD	6.70 ± 1.76	2.9 ± 3.07

Table. 18 Mean (±SD) seasonal variation of DMSP utilizers (DMSPU) and DMS utilizers (DMSU)

at Dona Paula bay

Variables	Spearman r	p value
TVC & Temperature	-0.554	< 0.05
TVC & TOC	0.835	< 0.001
TVC & DMSP	0.566	< 0.05
TVC & pH	-0.608	< 0.05
TVC & CFU	0.89	< 0.001
TVC & DMSPU	0.874	< 0.001
TVC & DMSU	0.618	< 0.01
CFU & Temperature	-0.65	< 0.01
CFU & pH	-0.569	< 0.05
CFU & TOC	0.775	< 0.001
CFU & DMSPU	0.896	< 0.001
DMSPU & pH	-0.665	< 0.01
DMSPU & TOC	0.802	< 0.001
DMSPU & DMSP	0.67	< 0.01
DMSU & Salinity	-0.613	< 0.01
DMSU & TOC	0.669	< 0.01

Table. 19 Spearman correlation coefficients of Biological variables during annual study at Dona Paula bay

Principal Component Analysis

Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Variation
1	8.35	63.9	63.9
2	1.26	9.6	73.6
3	1.03	7.9	81.4

Eigenvectors

(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3
Temperature	0.031	0.008	-0.031
pH	0.005	-0.003	-0.005
Salinity	0.020	-0.036	-0.026
DO	0.003	-0.027	0.047
Chl a	-0.049	0.166	-0.109
Phaeopigments	0.020	0.043	0.008
Nitrate	-0.033	-0.276	0.432
Nitrite	0.013	-0.086	0.217
Phosphate	0.041	-0.058	-0.094
Primary productivity	-0.115	0.126	-0.004
DMS	-0.090	0.354	0.037
DMSP	-0.166	0.088	0.236
TOC	-0.120	-0.047	0.179
Phytoplankton	0.001	0.739	0.063
TC	-0.127	-0.040	-0.568
TVC	-0.682	-0.012	-0.008
CFU	-0.591	-0.081	-0.175
DMSPU	-0.229	-0.128	-0.030
DMSU	-0.180	-0.069	0.504
Wind	-0.018	0.013	0.077
Flux	-0.116	0.394	0.183

Table. 20 Eigen values and vectors of all variables during the annual study

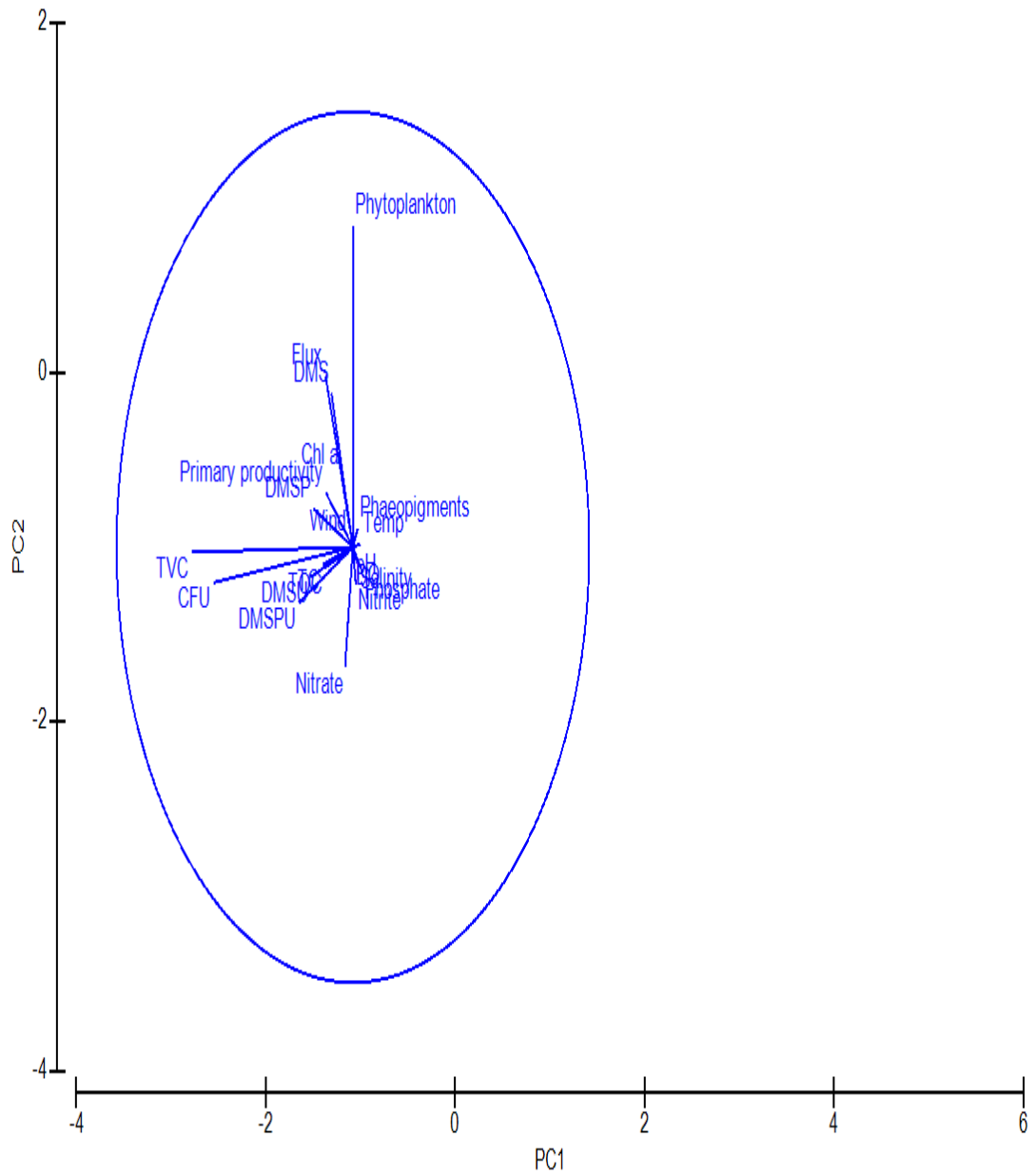


Fig. 35 The Principal Component Analyses of the variables measured during the annual study

4.2 Composition

4.2.1 Diatoms

4.2.1.1 Diurnal

The distribution of species showed temporal variation during the diurnal study. The dominant species were *Navicula* sp, *Bellorochea* sp, *Climacosphenia* sp and *Chaetoceros* sp which belonged to diatom community (Table 21). *Navicula* sp and *Bellorochea* sp were present throughout the study period, but the density varied at time intervals. *Thalassionema nitzschioides*, *Asteromphalus* sp, *Climacosphenia* sp, *Diploneis* sp, *Fragilaria* sp were more abundant from 19:00 to 07:00 hr. *Skeletonema* sp occurred at 07:00 hr only. Species like, *Pleurosigma* sp, *Odontella* sp, *Coscinodiscus* sp, and *Thalassiothrix* sp were present in the collection of 15:00 hr.

4.2.1.2 Annual

During the annual study, diatoms were the major group present. Among the diatoms, *Chaetoceros* sp, *Coscinodiscus* sp and *Thalassionema* sp were the dominant ones. During monsoon months, *Biddulphia* sp, *Chaetoceros* sp, *Coscinodiscus* sp, *Pleurosigma* sp were abundant in the community. The abundance of *Pleurosigma* sp, *Ditylum* sp, *Suriella* sp and *Chaetoceros* sp increased during post-monsoon months. During this season, presence of species like, *Streptotheca* sp, *Gyrosigma* sp, *Bacillaria* sp, *Triceratium* sp was observed whereas during the pre-monsoon

season other species such as *Thalassionema* sp, *Nitzschia* sp and *Pleurosigma* sp increased in density. Species like *Bacillaria* and *Triceratium* mostly occurred during the pre-monsoon season (Table 22).

4.2.2 Dinoflagellates

4.2.2.1 Diurnal

Among the dinoflagellates *Protoperidinium* sp and *Ceratium* sp were the dominant species. *Protoperidinium* sp was present throughout the sampling period, though the density increased at 19:00 hr (Table 21). It had the least density at 07:00 hr. *Ceratium furca* were present between 19:00 hr and 07:00 hr.

4.2.2.2 Annual

The dinoflagellates were low in density. Among them *Ceratium* sp, *Dinophysis* sp, *Noctiluca* sp and *Prorocentrum* sp were more abundant in the bay. Seasonally, dinoflagellates were more abundant during the post-monsoon season. Species like *Ceratium* sp, *Noctiluca* sp, *Prorocentrum* sp, and *Protoperidinium* sp were abundant during post – monsoon. *Noctiluca* sp had the highest abundance during the month of October and November. During the monsoon season, the dinoflagellates had the lowest population and were at minimal density. During pre-monsoon, the abundance of *Ceratium* sp and *Dinophysis* sp were high (Table 22).

4.2.3 Bacteria

Identification based on Biochemical tests

In the present study, 175 randomly selected from (quarter of a section) strains that grew rapidly on DMSP-containing plates were studied. All were found to be capable of growing on DMSP as sole carbon source. The strains purified were subjected to morphological, physiological and biochemical characterisation. The morphological characteristic of these strains are tabulated in Table 23. Majority of the colonies (53.7%) were irregular in shape and the rest were either punctiform (24.5 %) or circular (21.7%). They were mostly opaque and convex with 53.7% having undulate margins. Only a small group of the colonies were viscous (19.4%) whereas 77% of the colonies were butyrous and smooth (85.7%). Most of them were gram negative and motile with monotrichous flagella. Only 10% were non motile. A few of them were pigmented and some were sensitive towards penicillin. The biochemical characteristics showed that most of them were oxidase (92.5%), catalase positive (94.2%) and were able to oxidize glucose.

Biochemical characterisation and identification of the strains were carried out upto generic level. Based on the scheme of Oliver (1982), bacteria growing on DMSP plates were identified as *Pseudomonas* sp, *Xanthomonas* / *Flavobacterium* sp, *Vibrio* sp, *Moraxella* sp and *Bacillus* sp (Fig. 36). About 29% of DMSP strains

could not be classified according to this scheme (i.e. unidentified strains). Representatives from each of the above groups which grew well in DMSP on repeated sub culturing were tested for their catabolic potentialities.

Substrate utilisation

These fifty strains belonged to the genera *Pseudomonas* sp (34%), *Bacillus* sp (14%), *Xanthomonas / Flavobacterium* sp (10%), *Vibrio* sp (2%) and unidentified (40%) (Fig. 37). Though all the strains grew on all the tested substrates there were difference in the growth patterns. Sucrose served as a growth substrate for all strains. Other substrates where prolific growth occurred were in pyruvate, butyrate, lactic acid and succinic acid. However, very few strains showed growth on MPA and MMPA which were by products of DMSP metabolism. The multivariate analyses of the 50 strains based on their growth on substrates resulted in 8 groups (Fig. 38). The grouping depended mainly on their growth (OD) and number of substrates. Group 1 contains 17 strains which grew well in most of the substrates (14), thus being the most versatile group. In the second group, the growth was medium and the substrates utilized were only eight (Sucrose, pyruvate, butyrate, lactate, acetate, succinate, formate and methanol). The fourth group had 4 strains, and the OD towards the compounds with largest affinity was of the range 0.3 to 0.4. Groups 5 and 6 had 4 strains, showed affinity to other substrates compared to sucrose, and the highest response

was 0.2 – 0.3. The seventh group had 11 strains, which mostly grew on sucrose, pyruvate, lactate, butyrate and acetate, but the growth was low (OD ranged from 0.1 – 0.2). The eighth group had 7 strains and the affinity was limited to a few substrates only. Three strains were independent and did not form a group and dissimilarity was observed on their growth pattern on MPA and MMPA.

Multivariate analysis was performed to bring out the similarities in utilization pattern of the strains based on their identification. *Pseudomonas* sp (34%) emerged into 3 groups and a separate single strain at 80% similarity index (Fig. 39), The first group had 9 strains and all of them belonged to highly versatile group obtained in the above said analyses. The strains belonging to other groups showed low growth response to the supplied substrates. Among the seven strains of the *Bacillus* sp, 3 groups emerged with 85% similarity index (Fig. 40). These groups were neither versatile nor showed prolific growth. The strains belonging to *Xanthomonas / Flavobacterium* sp, were grouped into two, with 80% similarity (Fig. 41). One group contained 2 strains and they belonged to the versatile utilizers with prolific growth. The other group contained 3 strains, which were not highly responsive to all the substrates supplied. Among the unidentified strains, at 80% similarity 3 groups emerged (Fig. 42). The first group contained 8 strains, which belonged to the versatile utilizers with prolific growth. The other two groups were slow growers and did not show marked

affinity to the substrates supplied. Five strains were chosen from these groups and studied in detail.

4.3 Studies on Five DMSP Utilizers

4.3.1 Strains #1, #2 and #3

4.3.1.1 Characteristics

All three strains were gram-negative rods shaped to circular, 0.6 - 0.7 x 1.0 - 2.0 μm . Microscopically they appear arranged singly or in pairs. Colonies are smooth, convex, pearly, glistening, greyish-white butyrous and differ in size. They were negative for amylase, lipase and gelatinase. Among the 95 carbon sources in the Biolog system, for the strains #1, #2 and #3 the following characteristics were negative - assimilation of D and L Arabinose, mannose, mannitol, N-acetylglucosamine, maltose, glycerol, erythritol, ribose, L and D xylose, adonitol, rhamnose, methyl β - D-xyloside, inositol, sorbitol, methyl α -D glucoside, lactulose, saccharose, trehalose, raffinose, starch, glycogen and xylitol. Tween 40 and 80 was utilized by these strains (Table. 24).

FAME analysis

The fatty acid analysis of these strains is as given in the table. The characteristic fatty acids present in strains #1, #2 and #3 were C_{16:0} - 33.3, 33.12 & 33.58 % ; C_{16:1 ω 7C} - 26.65, 27.79 & 27.15 %, C_{17:0 CYCLO} - 8.7, 7.0, 8.16 % respectively (Table 25).

16S rDNA analyses

Strain # 1 The sequences for the strain is as follows -

Forward sequence

CTTCTTCGTCAGTTCGGTGCATGCTTCCACGTTACCTCATCTTGACATGT
CTGGAATGC

CGAAGAGATTTGGCAGTGCTCGCAAGAGAACCGGAACACAGGTGCTGCATG
GCTGTCGTCAGCTCGTGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTGTCAATTAGTTGCTACGAAAGGGCACTCTAATGAGACTGCCGGTG
ACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGG
TAGGGCTTCACACGTCATACAATGGTCCGGACAGAGGGCAGCCAACCCGC
GAGGGGGAGCCAATCCCAGAAACCCGATCGTAGTCCGGATCGCAGTCTGC
AACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGTCCG
CGGTGATCCTTTTCTCCCGGCCAAAAAAATTTCCCGGGCCAAGAAAAAACT
TTCCCGGG

Reverse sequence

GCATTGTGCTGATGCCGACTGCTTTGGGCACCGACTTCACGCAGTCGAGTT
GCAGACTGCGATCCGGACTACGATCGGGTTTCTGGGATTGGCTCCCCCTCG
CGGGTTGGCTGCCCTCTGTCCCGACCATTTGTATGACGTGTGAAGCCCTACC
CATAAGGGCCATAGAGGACTTGACGTCAATCCACCTTCTCCGGTTTGTCA
CCGGCAGTCTCATTAGAGTGCCCTTTCGTAGCAACTAATGACAAGGGTTGC
GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCC
ATGCAGCACCTGTGTTCCGGTTCTCTTGCGAGCACTGCCAAATCTCTTCGCA
TTCCAATGTCACGAGAAGGTAAGGTTTTTCGCGGTGGATCAATTAACCACAT
GCTCCACGCTTGTGCAAACAGGAAAAAAAAAAAA

Strain # 2 The sequences for the strain is as follows -

Forward

AACGCGTTAGTTCGATGCATACCTTCCACGTTACCTCACCTTGTACATGTC
TGAGAATGCCGAAGAGATTTGGCAGTGCTCGCAAGAGAACCGGAACACAGG
TGCTGCATGGCTGTGTCGTCAGCTCGTGTGTCGTGAGATGTTGGGTTAAGTCCCG
CAACGAGCGCAACCCTTGTCAATTAGTTGCTACGAAAGGGCACTCTAATGAGA

CTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGG
CCCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGGACAGAGGGCAG
CCAACCCGCGAGGGGGAGCCAATCCCAGAAACCCGATCGTAGTCCGGATC
GCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGGATC
AGCATGTCGCGGTGATCCTTTTTCCCGGGCA

Reverse

ACGTTGTGCTGATCCGCGATTACTTGGGGCTCCGACTTCACGCAGTCGAGT
TGCAGACTGCGATCCGGACTACGATCGGGTTTCTGGGATTGGCTCCCCCTC
GCGGGTTGGCTGCCCTCTGTCCCAGCATTGTATGACGTGTGAAGCCCTAC
CCATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTG
ACCGGCAGTCTCATTAGAGTGCCCTTTCGTAGCAACTAATGACAAGGGTTGC
GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCC
ATGCAGCACCTGTGTTCCGGTTCTCTTGCGAGCACTGCCAAATCTCTTCGGC
ATTCCAGACATGTCAAGGGTAGGTAAGGTTTTTCGCGTTGCATCGAATTAAT
CCACATGCTCCACCGCTTGTGCA

Strain #3

Forward

CGCTTCTTCGACAGGTCCGGTGCATACCTTCCCGGGTCACCTATCACTTGACA
TGGCTGGAATGCCGAAGAGATTTGGCAGTGCTCGCAAGAGAACCGGAACAC
AGGTGCTGCATGGCTGTCGTACGCTCGTGTGAGATGTTGGGTTAAGTC
CCGCAACGAGCGCAACCCTTGTCATTAGTTGCTACGAAAGGGCACTCTAAT
GAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTC
ATGGCCCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGGACAGAGG
GCAGCCAACCCGCGAGGGGGAGCCAATCCCAGAAACCCGATCGTAGTCCC
GGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGC
GGATCCACATTGTCGCGGTGAATACTTTCCCGGGACA

Reverse

TTTCGTTGTGCTGATGCTCACTGCTTGGGGCACCGACTTCACGCAGTCGAG
TTAGCAGACTGCGATCCGGACTACGATCGGGTTTCTGGGATTGGCTCCCCC
TCGCGGGTTGGCTGCCCTCTGTCCCAGCATTGTATGACGTGTGAAGCCCT
ACCCATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCGGTTT
TCACCGGCAGTCTCATTAGAGTGCCCTTTCGTAGCAACTAATGACAAGGGTT

GCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAG
CCATGCAGCACCTGTGTTCCGGTTCTCTTGCGAGCACTGCCAAATCTCTTCG
GCATTCCAGACATGTCAAGGGTAGGTAAGGTTTTTCGCGTTGCATCGAATTA
ATCCACATGCTCCACCGCTTGTGCAA

The minimal temperature for growth was 18°C for strain# 1 whereas for strains #2 & #3 it was 22 °C. The maximum temperature in which growth occurred was 40°C for strain#1 and 42°C for strains #2& #3. They differed in their NaCl requirements. Strains #1 & #3 had an optimum NaCl requirement of 3% whereas strain #2 had the optimum at 3.5%. The maximal NaCl concentration at which they could grow was 10% for strains #1 & #3, whereas for strain #2, it was 15% (Table. 26a).

Sequences were inspected, corrected and assembled into a single consensus sequence for the strains. After that, the sequences were examined with the BLAST search alignment tool comparison software (BLAST) (<http://www.ncbi.nlm.nih.gov/>

BLAST/) to detect the closest bacterial group to each strain from the GenBank database.

The strain #1 had an SI of 100% with *Bordetella petrii*.

The strain #2 had an SI of 99% with *Bordetella petrii*

The strain #3 had an SI of 98%with *Bordetella petrii*

These three strains were placed under

Phylum: Proteobacteria

Class: Beta : Proteobacteria

Order: Burkholderiales

Family: Alcaligenaceae

Genus: *Bordetella*

Species: *Petrii*

4.3.1.2 Chemotaxis

Experiments were designed to investigate the chemotactic response of the strains towards DMSP. These strains were also checked for their response to sucrose, cysteine and acrylic acid (Table 26 b). Unlike *Bordetella petrii* #1 and #2, *Bordetella petrii* #1 responded to all the substrates. *Bordetella petrii* #2 was active towards sucrose only. *Bordetella petrii* #3 was active towards sucrose as well as cysteine.

There was a marked difference in their behaviour when these strains were tested for their chemotactic ability at varying pH and concentration to DMSP. In order to assess the response in highly acidic environment due to senescence or the release of acrylic acid pH was varied. Also it is known that DMSP degrades in alkaline conditions. *Bordetella petrii*#1 responded to higher range of DMSP at pH 2, whereas at pH 7.5, it was sensitive to lower ranges of

DMSP concentration. *Bordetella petrii* #2 fared well at pH 7.5, being responsive from pico to micromolar range. At pH 6 & 7.5, its response level enhanced and more than 50% of the cells were active (Table 26 c). *Bordetella petrii* #3 showed a marked variation in its chemotactic ability in comparison to other two strains. It responded well to all the ranges of concentration at all pH, except at pH 6 whereas the pico molar level failed to elicit any response (Table. 26 c).

4.3.1.3 DMSP utilization

These strains were subjected for further tests on DMSP. The initial kinetics of DMSP utilization showed considerable variability among the tested strains. The *Bordetella petrii* #1 showed negligible growth in NaCl concentrations of 1.0 and 1.5 ‰ compared to 2.0, 3.5, 4.0 and 6.0 ‰. The highest growth was observed at 3.5 and 6.0 ‰ of NaCl. Likewise, when the pH of the media was altered, it was observed that the strain exhibited enhanced growth at pH 6 compared to pH 7.5 and 9. When the temperature was modified, growth was boosted at 37°C than 10°C or 28 °C (Figs. 43, 44 & 45).

Bordetella petrii #2 showed a gradation in its growth according to increasing salinity. As the salinity gradient increased, growth also increased, except at 5.0 ‰. The highest growth was measured at 3.5 ‰ of NaCl. Likewise, when the pH of the media was amended, the strain exhibited enhanced growth at pH 6 compared to pH 7.5

and 9. Upon adjusting the temperature, growth was visible both at 10°C and 37°C compared to 28°C (Figs. 46, 47, & 48).

It was observed that, for the *Bordetella petrii* # 3, NaCl concentrations of 3.5 % was more favourable for growth compared to the other concentrations tested. The most favourable pH for enhanced growth was pH 6 which was same for other two strains also. It had mesophilic characteristics, as growth was more evident at 28 °C and 37°C, than at 10 °C (Figs. 49, 50,& 51).

4.3.2 Strain #4

4.3.2.1 Characteristics

Colonies 2-3 days old are circular, colourless and transparent; in older cultures, they turn opaque. Aerobic and microaerophilic, Non-spore-forming rods, 0.6 – 0.8 x 1.6 – 2.5 µm when grown in DMSP supplemented mineral medium. Oxidase and catalase positive. Actively degrades Tween 80, catalase and oxidase positive, negative for agarase, amylase and gelatinase. Nitrate is reduced to nitrite.

The strain could utilise only a few simple organic compounds as carbon and energy source (formate, acetate, propionate, methyl – pyruvate and α - ketoglutarate) which matches the description given by Bergey's manual. Among the 95 carbon sources in the Biolog system, the strain 4, was positive for Tween 40 and 80, methyl pyruvate, mono-methyl succinate, acetic acid, citric acid, β hydroxybutyric acid, γ-hydroxybutyric acid, phydroxyphenylacetic

acid, lactic acid, propionic acid, bromosuccinic acid, malonic acid, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, inosine and 2, 3 butanediol (Table. 27).

FAME analysis

The fatty acid profile of the strain is as given in table. Characteristic principal fatty acids of strain were C_{15:0} - 0.54%, C_{16:0} – 7.84%, C_{16:1 ω 7c} – 9.35% and C_{18:1 ω 7c} – 1.2% (Table. 28).

16S rDNA analyses.

Forward

```
AAACTTTATTTCGATGCACGCGAAGAACCTTACCAGGTCTTGACATCCTGCGA
ACTTTCTAGAGATAGATTGGTGCCTTCGGGAGCGCAGTGACAGGTGCTGCA
TGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAG
CGCAACCCTTGTCTTAGTTGCCAGCACTTCGGGTGGGAAGTCTAGGGAGA
CTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGG
CCCTTACGGCCTGGGCTACACACGTGCTACAATGGTTGGTACAGAGGGTTG
CGAAGTCGCGAGGCGGAGCTAATCTCTCAAAGCCAATCGTAGTCCGGATTG
GAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCGGATCA
AAATGCCGCGGTGAATACGTTTCCCGGGCA
```

Reverse

```
ACGTCATTCTGATCCGCGATTACTAGCGATTCCGACTTCATGGAGTCGAGTT
GCAGACTCCAATCCGGACTACGATTGGCTTTGAGAGATTAGCTCCGCCTCG
CGACTTCGCAACCCTCTGTACCAACCATTGTAGCACGTGTGTAGCCCAGGC
CGTAAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTGTCA
CCGGCAGTCTCCCTAGAGTTCCCACCCGAAGTGCTGGCAACTAAGGACAAG
GGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGACACGAGCTGACG
ACAGCCATGCAGCACCTGTCACTGCGCTCCCGAAGGCACCAATCTATCTCT
AGAAAGTTTCGAGGATGTCAAGGCCTGGTAAGGTTCTTCGCGTTGCATCGA
ATTAAACCACATGCTCCCCGCTTGTGCACAACA
```

The optimum temperature was 28°C for the strain and the minimum temperature was 18 °C. The maximum temperature at which growth occurred was 40 °C. The strain required NaCl for its growth. The optimal NaCl concentration was 3% whereas the maximum was 7.5% (Table. 29a). The sequences obtained by 16s rDNA analyses is as given below.

Sequences were inspected, corrected and assembled into a single consensus sequence for the strain. After that, the sequences were examined with the BLAST search alignment tool comparison software (BLAST) (<http://www.ncbi.nlm.nih.gov/BLAST/>) to detect the closest bacterial group to each strain from the GenBank database.

The strain#4 had an S.I - 98% to *Alcanivorax dieselolei*

The Bergey's manual placed the strain # 4 into

Phylum: Proteobacteria

Class: Gamma- Proteobacteria

Order: Oceanospirillales

Family: Alcanivoraceae

Genus: *Alcanivorax*

Species: *dieselolei*

4.3.2.2 Chemotaxis

When assessed for chemotactic affinity, *Alcanivorax dieselolei* was active towards only sucrose among other compounds (Table. 29b). The strain exhibited high chemotactic affinity towards DMSP at all the pH and concentrations of DMSP supplied. At pH 2 and pH 6, it responded to all the concentrations of DMSP supplied. Chemotactic attraction towards DMSP at picomolar range at pH 2 & 6 was shown by 100% of cells, whereas more than 60% of cells responded towards nanomolar range at the same range of pH. The response towards the highest concentration of DMSP did not vary significantly with pH. However at pH 7.5, the strain showed a different response level towards the lower concentrations. The cells were not attracted to picomolar levels but to higher concentrations only at pH 7.5. Few (18%) cells responded to micromolar range of DMSP, and fewer cells (3%) responded to nanomolar range of DMSP (Table. 29c).

4.3.2.3 DMSP Utilization

The strain *Alcanivorax dieselolei* demonstrated variation in growth at different concentration of NaCl. The growth was measured in terms of biomass (protein). Growth was the highest at 6.0 % with a biomass of $16.8 \mu\text{g l}^{-1}$. At 60ppt, DMSP utilized was $495 \mu\text{M}$. Also, enhanced growth was recorded at pH 6 compared to pH 7.5 and 9. At pH 6 the biomass accumulated was $42 \mu\text{g l}^{-1}$ whereas at pH 7.5

and 9, it was 19.5 and 12 $\mu\text{g l}^{-1}$, respectively. The DMSP utilized was higher at pH 6 and 7.5 (450 μM) compared to pH 9 (430 μM). During the experiments, the temperature of 37°C was favourable for growth than 10°C or 28 \pm 2 °C. At 37 °C the biomass accumulated was 23.2 $\mu\text{g l}^{-1}$, whereas at 10 and 28 \pm 2 °C, the growth was 12 and 14 $\mu\text{g l}^{-1}$. The DMSP utilized (460 μM) was highest at 10 °C and the least (360 μM) at 28 °C (Figs. 52, 53 & 54).

4.3.3 Strain #5

4.3.3.1 Characteristics

Rods containing endospores, 0.5 – 1.0 x 1.4 – 2.8 μm when grown in DMSP supplemented mineral medium. Colonies 2-3 days old are irregular and creamish in colour. They were aerobic, oxidase and catalase positive, nitrate reduced to nitrite. It tested positive for amylase and gelatinase. Other characteristics are given in the species description.

Among the 95 carbon sources in the Biolog system, the strain 5 was positive for erythritol, _D fructose, adonitol, _L fucose, _Draffinose, α aminobutyric acid, inosine, _L-rhamnose, _Dsorbitol, quinic acid, saccharic acid, proline, serine (Table. 30).

FAME analyses

The fatty acid profile of the strain is as given in the table. The characteristic fatty acid present were $\text{C}_{12:0}$ – 0.22%, $\text{C}_{14:0}$ – 5.66%,

C₁₆:0 – 33.71%, C_{16:1 ω 7C/15 iso 2OH} (Sum in feature 3, C_{ANTEISO 17:1 ω 9C} - 4.29 %, C_{18:0} – 4.29%) (Table. 31).

16S rDNA analyses

The forward and reverse sequences of the strain are as given.

Forward

```
ACTCTTTATTTCGAGCACGCGGGACCTTACCATGTGTCTTGACATCTCCTGAC
AACCCTAGAGATAGGGCGTTCCCTTCGGGGGACAGGATGACAGGTGGTG
CATGGTTGTCGTCAGCTCGTGTGTCGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTGATCTTAGTTGCCAGCATTTCAGTTGGGCACTCTAAGGTGA
CTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCC
CCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGC
GAAACCGCGAGGTTAAGCGAATCCATAAAACCATTCTCAGTTCGGATTGCA
GGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCAGC
ATGCCGCGGTGATAAGTTTTCTCCGGGCCATGGGGGCGCGATGAGAGCTG
TCCGCTTTTTTATTATTCTCTCAAACAACCTG
```

Reverse

```
TTTTTCGTCATGCTGATCCGCGATTACTAGCGATTCCGGCCTTCATGCAGGC
GAGTTGCAGCCTGCAATCCGAACTGAGAATGGTTTTATGGGATTCGCTTAAC
CTCGCGGTCTCGCAGCCCTTTGTACCATCCATTGTAGCACGTGTGTAGCCC
AGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCTCCGGTTTG
TCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCA
AGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGA
CGACAACCATGCACCACCTGTCATCTGTCCCCGAAGGGGAACGCCCTAT
CTCTAGGGTTGTCAGGAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTT
CGAATTAACACATGCTCCACCGCTTGTGCACGCCC
```

The optimum temperature for growth for the strain 5 was 32 °C. The minimal temperature was 20 °C and the maximal temperature was 42 °C. The strain required NaCl for its growth. The optimum NaCl concentration was 3% and maximum NaCl concentration was 7.5% (Table.32 a).

Sequences were inspected, corrected and assembled into a single consensus sequence for each phylotype. After that, the sequences were examined with the BLAST search alignment tool comparison software (BLAST) (<http://www.ncbi.nlm.nih.gov/BLAST/>) to detect the closest bacterial group to each strain from the GenBank database.

The strain #5 had an S.I - 96% with *Bacillus sp.*

The strain #5 belongs to

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Bacillaceae

Genus: *Bacillus*

4.3.3.2 Chemotaxis

The *Bacillus sp.* was chemotactically active towards sucrose as well as cysteine but not towards acrylic acid (Table.32 b).

The strain had a graded response towards different concentration of DMSP. At pH 2, *Bacillus sp.* was active towards higher concentrations of DMSP. At micromolar concentration of DMSP, 31% of cells were active, whereas at nanomolar levels 88% of cells were found to be actively moving towards DMSP and there was no response at picomolar levels of DMSP. When supplied with

micromolar and nanomolar levels of DMSP at pH 6 and pH 7.5, more than 55 % of cells were responsive. The response level was higher for all the supplied concentrations of DMSP at pH6 (Table.32 c).

4.3.3.3 DMSP Utilization

DMSP Utilization

Bacillus sp. was able to grow in all the NaCl concentrations, pH and temperature range supplied. There were variations in the growth range to the different NaCl concentrations supplied. At 1.0 %, the growth was $18.6 \mu\text{g l}^{-1}$, whereas at 1.5 and 2.0 % it was 9.4 and $9.9 \mu\text{g l}^{-1}$. The biomass accumulated was higher at 1.0 %, compared to 1.5 and 2.0 %. Similarly at 1.0 %, the DMSP utilization was higher (400 μM) compared to 1.5 and 2.0 % (325 and 350 μM respectively). At 3.5, 4.0 and 6.0 %, the biomass and DMSP utilization were not significantly different. The DMSP was utilized completely at 3.5 and 4.0 %, though at 6.0 % the utilization was 490 μM of DMSP. Variation in growth was observed when according to the range of pH supplied. The highest growth was observed at pH 6 with the accumulation of $34 \mu\text{g l}^{-1}$ of protein. The DMSP utilization was also high at pH 6 (470 μM). At pH 7.5 and 9, the DMSP utilized was 400 and 350 μM . The DMSP utilization was high at all the temperature supplied, but the growth rate was different (10, 28 and 37°C , 450 - 480 μM). The protein measured at 10, 28 and 37°C was 12.7, 32 and $33 \mu\text{g l}^{-1}$ (Figs 55, 56 & 57).

SI No	Species	15:00 hr	19:00 hr	06:00 hr	07:00 hr	13:00 hr
Diatoms						
1	<i>Chaetoceros sp</i>	240	80	-	-	-
2	<i>Coscinodiscus sp</i>	80	800	-	-	-
3	<i>Navicula sp</i>	7600	37360	80720	16240	15440
4	<i>Pleurosigma sp</i>	400	-	-	-	-
5	<i>Skeletonema sp</i>	-	-	-	80	-
6	<i>Thalassionema sp</i>	-	-	-	-	80
7	<i>Thalassionema nitzschioides</i>	-	160	80	80	-
8	<i>Bellerochea sp</i>	24320	6080	4480	830400	88560
9	<i>Thalassiothrix sp</i>	160	-	-	-	-
10	<i>Dityllum brightweii</i>	80	-	-	-	-
11	<i>Nitzchia sp</i>	240	160	-	-	-
12	<i>Odentella sp</i>	160	-	-	160	-
13	<i>Rhizosolenia sp</i>	-	80	-	-	-
14	<i>Fragilaria sp</i>	-	-	880	80	240
15	<i>Triceratium sp</i>	80	80	-	-	-
16	<i>Asteromphalus sp</i>	-	80	80	-	-
17	<i>Climacosphenia sp</i>	-	1440	400	800	1360
18	<i>Diploneis sp</i>	-	720	1120	720	480
19	<i>Coretron sp</i>	-	-	80	-	-
Dinoflagellates						
20	<i>Protoperidinium sp</i>	240	1040	160	80	240
21	<i>Ceratium furca</i>	-	80	-	80	-
22	Unidentified sp	50	70	100	150	140
Total no: of cells		33650	48230	88100	848870	106540

Table. 21 The phytoplankton distribution at Dona Paula bay during diurnal study.

Sl No	Species	April	May	June	July	August	September	October	November	December	January	February	March	April
Diatoms														
1	<i>Chaetoceros sp</i>	-	25	-	7200	-	-	-	-	3560	160	-	-	-
2	<i>Coscinodiscus sp</i>	540	50	1440	5120	150	50	1250	685	600	1120	1280	400	640
3	<i>Pleurosigma sp</i>	-	50	-	1040	-	-	250	2223	600	680	1360	-	-
4	<i>Navicula sp</i>	700	-	-	240	-	-	500	-	1200	160	2880	-	800
5	<i>Bellorochea sp</i>	-	-	-	-	-	-	-	-	700	-	-	-	-
6	<i>Thalassionema sp</i>	12500	175	2736	-	-	250	-	-	120	-	480	80	12320
7	<i>Thalassiothrix sp</i>	400	-	1872	-	-	-	-	-	-	120	-	-	400
8	<i>Ditylum sp</i>	100	-	576	800	-	-	-	1390	-	40	240	-	80
9	<i>Nitzschia sp</i>	-	-	144	400	-	-	-	-	160	120	1440	1760	-
10	<i>Rhizosolenia sp</i>	-	200	0	720	-	850	-	278	-	320	-	-	-
11	<i>Fragillaria oceanica</i>	-	-	144	-	-	-	-	-	-	-	-	-	-
12	<i>Eucampia sp</i>	-	-	-	-	-	50	-	-	40	200	160	-	-
13	<i>Hemialus sp</i>	-	25	-	-	-	-	-	973	40	160	80	-	-
14	<i>Biddulphia sp</i>	-	50	1080	1280	-	50	1000	-	280	120	160	80	-
15	<i>Leptocylindrus sp</i>	-	-	-	-	-	1000	-	-	-	-	480	-	-
16	<i>Melosira sp</i>	-	-	-	320	-	100	-	-	-	160	0	-	-
17	<i>Suriella sp</i>	250	-	-	240	1100	-	-	1945	120	-	960	-	240
18	<i>Skeletonema sp</i>	240	-	504	960	-	-	-	140	-	160	-	-	240
19	<i>Planktoniella sp</i>	-	-	-	-	-	-	-	-	-	440	-	80	-
20	<i>Bacillaria sp</i>	-	25	-	-	-	-	-	418	-	320	160	400	-
21	<i>Triceratium sp</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
22	<i>Streptotheca sp</i>	200	-	-	-	-	-	-	833	-	40	-	-	160
23	<i>Striatella sp</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
24	<i>Asterionella sp</i>	-	-	144	1120	-	-	-	-	-	-	-	-	-
25	<i>Halosphaera sp</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
26	<i>Gyrosigma sp</i>	-	-	1008	-	-	-	-	-	40	-	-	-	-

27	<i>Amphipora sp</i>	-	-	-	-	-	-	-	-	-	-	-	-	
28	<i>Detonula sp</i>	-	-	-	-	850	-	-	-	-	-	-	-	
29	<i>Hyalodiscus sp</i>	-	-	-	-	-	-	-	-	-	-	-	-	
30	<i>Thalassiosira sp</i>	-	50	-	160	350	200	-	-	-	-	-	-	
Dinoflagellates														
31	<i>Ceratium sp</i>	-	-	-	560	-	-	-	140	320	200	-	720	0
32	<i>Dinophysis sp</i>	70	-	-	-	-	-	-	-	-	200	-	-	80
33	<i>Noctiluca sp</i>	-	-	-	-	-	-	500	555	40	-	-	-	-
34	<i>Prorocentrum sp</i>	-	25	-	80	-	-	-	-	-	-	-	-	-
35	<i>Peridinium sp</i>	-	-	-	-	-	-	-	-	80	120	-	-	-
Other algae														
36	<i>Trichodesmium sp</i>	-	-	25	-	40	-	-	-	-	-	-	-	-
37	<i>Cocosphere sp</i>	-	-	-	-	-	-	-	140	-	-	-	-	-
38	<i>Laudicia sp</i>	-	-	432	-	-	-	-	-	-	40	-	-	-
39	<i>Silicoflagellates sp</i>	-	-	-	-	-	-	-	-	-	120	-	-	-
40	<i>Schodrella sp</i>	-	25	-	-	-	-	-	-	-	-	-	-	-
41	Unidentified algae	25	-	1728	-	30	-	40	140	50	160	40	40	-
Total no: of cells		15025	700	11833	20240	2520	2550	3540	9860	7950	5160	9720	3560	14960

Table. 22 The phytoplankton distribution at Dona Paula bay during annual study

Morphological Characteristics		Percentage
Shape	Punctiform	24.57
	Irregular	53.71
	Circular	21.71
Margin	Entire	46.28
	Undulate	53.71
Elevation	Convex	88.00
	Flat	08.57
Opacity	Opaque	88.00
	Transparent	10.85
Texture	Viscous	19.42
	Butyrous	77.14
Surface	Mucoid	12.00
	Smooth	85.71

Table. 23 Morphological characteristics of the colonies of DMSPU

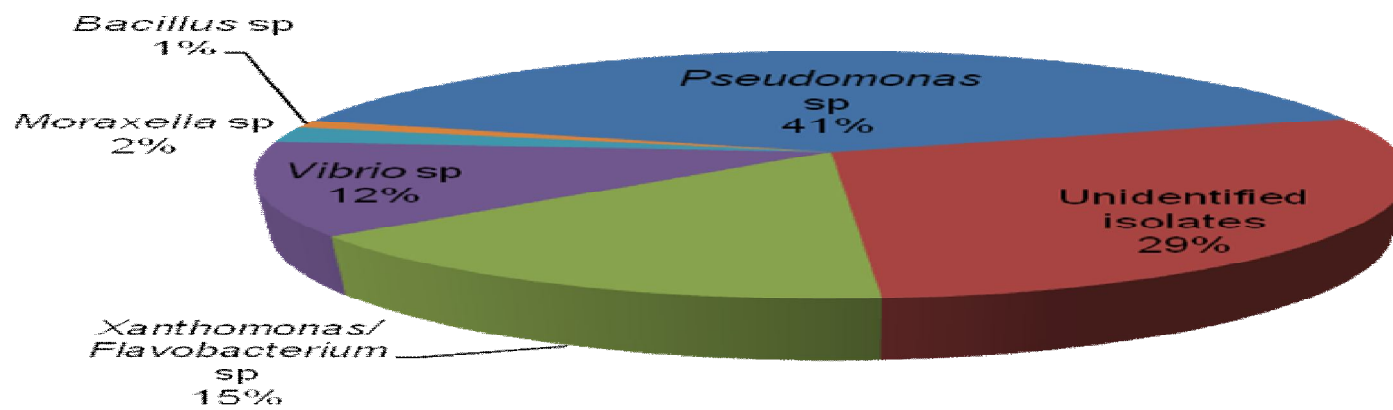


Fig. 36 The generic composition of DMSPU

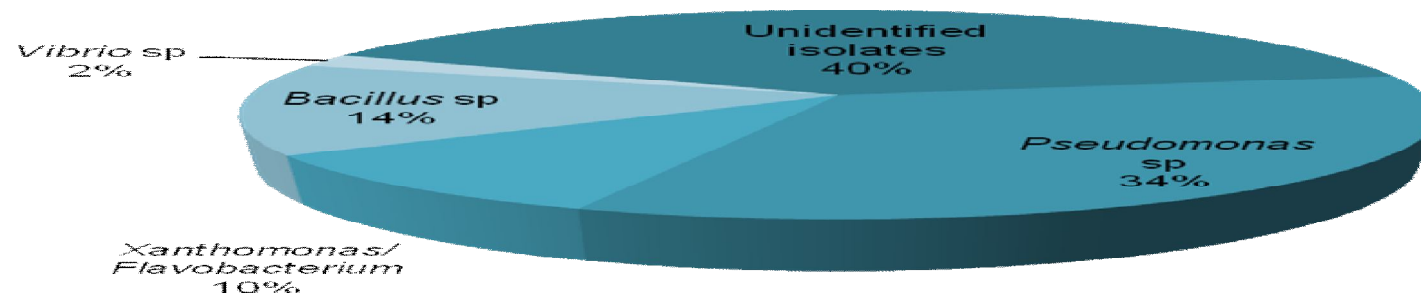


Fig. 37 The generic composition of the 50 isolates based on substrate utilization

Sl no	Substrates	<i>Bordetella petrii</i> (#1)	<i>Bordetella petrii</i> (#2)	<i>Bordetella petrii</i> (#3)
1	Dextrin	+	—	+
2	Tween 80	+	—	—
3	N-Acetyl D Galactosamine	+	+	—
4	N-Acetyl D Galactosamine	+	—	+
5	Adonitol	—	+	+
6	L-Arabinose	+	—	+
7	D-Arabitol	+	+	—
8	D-Cellobiose	+	+	—
9	I-Erythritol	+	—	—
10	D-Fructose	+	+	—
11	L-Fucose	+	+	—
12	D-Galactose	—	+	—
13	Gentobiose	+	+	—
14	Apha D Glucose	+	+	—
15	Inositol	+	—	—
16	Alpha D Lactose	+	—	—
17	Lactulose	+	+	—
18	D-Mannitol	—	+	+
19	D-Mannose	—	+	—
20	D-Raffinose	—	+	—
21	D-Sorbitol	+	—	+
22	D-Treahlose	+	—	+
23	Pyruvic Acid Methyl Ester	—	+	—

24	Succinic Acid Mono Methyl Ester	—	+	—
25	Acetic Acid	—	+	+
26	Cis Aconitic Acid	—	+	—
27	Cirtic Acid	—	+	—
28	Formic Acid	—	+	+
29	D Galacturonic Acid	+	+	—
30	D Glucuronic Acid	+	—	+
31	P-Hydroxy Phenylacetic Acid	—	—	+
32	Alpha Keto Butyric Acid	+	+	—
33	Alpha Keto Glutaric Acid	+	+	—
34	Apha Keto Valeric Acid	+	—	—
35	D,L,Lactic Acid	+	+	—
36	Propionic Acid	+	+	—
37	Sebacic Acid	+	+	—
38	Succinic Acid	—	+	+
39	Bromosuccinic Acid	—	+	—
40	Succinamic Acid	+	+	—
41	Glucuronamide	+	—	+
42	L Alanimide	—	—	+
43	L-Alanine	+	+	—
44	L Asparagine	+	+	—
45	L-Glutsmic Acid	+	+	—
46	Glycyl L- Glutamic Acid	+	+	—
47	L-Histidine	—	—	+
48	Hydroxy L Proline	—	+	—

49	L Leucine	—	+	+
50	L Ornithne	—	+	—
51	L Proline	+	+	—
52	L Serine	+	+	—
53	D,L Carnitine	+	+	—
54	Alpha Amino Butyric Acid	—	+	+
55	Urocanic Acid	—	—	+
56	Inosine	—	+	+
57	Uridine	—	—	+
58	Thymidine	+	—	+
59	Putrescine	—	—	+
60	2-Aminoethanol	—	—	+
61	2,3 Butanediol	—	+	+
62	Glycerol	—	+	—
63	D,L-Alpha Glycerol Phosphate	—	+	+
64	Alpha D Glucose 1 Phosphate	+	+	—
65	D Glucose 6 Phosphate	—	+	—

Table. 24 Carbon utilization patterns of the 3 *Bordetella petrii* strains

Fatty Acids	<i>Bordetella petrii</i> (#1) (%)	<i>Bordetella petrii</i> (#2) (%)	<i>Bordetella petrii</i> (#3) (%)
C ₁₂ :0	0.25	0.27	0.19
C ₁₂ :0 ALDE			0.37
C ₁₂ :0 2OH	0.09		
C ₁₂ :0 3OH	0.18	0.18	0.2
C ₁₄ :0	5.61	5.65	5.69
C ₁₄ :0 2OH	2.15	2.22	2.11
C ₁₄ :1 ω 5C	0.2		
C ₁₄ :0 3 OH/16:1 ISO 1	6.25	6.24	6.41
(Sum in feature 2)			
C ₁₅ :0	0.22	0.15	0.19
C ₁₆ :0	33.33	33.12	33.58
C ₁₆ :1 ω 9C		0.44	0.23
C ₁₆ :1 ω 7C/15 iso 2OH	26.65	27.79	27.15
(Sum in feature3)			
C ₁₆ :0 3OH	0.47	0.42	
C ₁₇ :0CYCLO	8.79	7	8.16
C ₁₈ :1 ω 9c	0.65	1.12	0.6
C ₁₈ :1 ω 7c	9.49	10.03	9.58
C ₁₈ :0	4.5	4.82	4.42

Table. 25 Fatty acids profile of the *Bordetella petrii* strains

Strains	Characteristics					
	Temperature for growth (°C)			Growth in NaCl (%)		
	Minimal	Optimum	Maximum	Minimal	Optimum	Maximum
<i>Bordetella petrii</i> (#1)	18	37	40	0.5	3	10
<i>Bordetella petrii</i> (#2)	22	37	42	0.5	3.5	15
<i>Bordetella petrii</i> (#3)	22	37	42	0.5	3	10

Table. 26 a) Cell growth at different temperatures and NaCl concentrations

Strains	Sugar	Cysteine	Acrylic acid
<i>Bordetella petrii</i> (#1)	+	+	+
<i>Bordetella petrii</i> (#2)	+	+	-
<i>Bordetella petrii</i> (#3)	+	-	-

Table. 26 b) Chemotactic response towards Sucrose, Cysteine and Acrylic acid

Strains	pH 2			pH 6			pH 7.5		
	Conc of DMSP (percentage of responsive cells)								
	300µM	60nM	10pM	60µM	12nM	2pM	60µM	12nM	2pM
<i>Bordetella petrii</i> (#1)	2	0	0	2	1	0	0	9	1
<i>Bordetella petrii</i> (#2)	58	55	4	57	52	0	82	37	20
<i>Bordetella petrii</i> (#3)	0	25	25	19	23	25	0	17	4

Table. 26 c) Chemotactic responses towards DMSP at different pH and DMSP concentrations

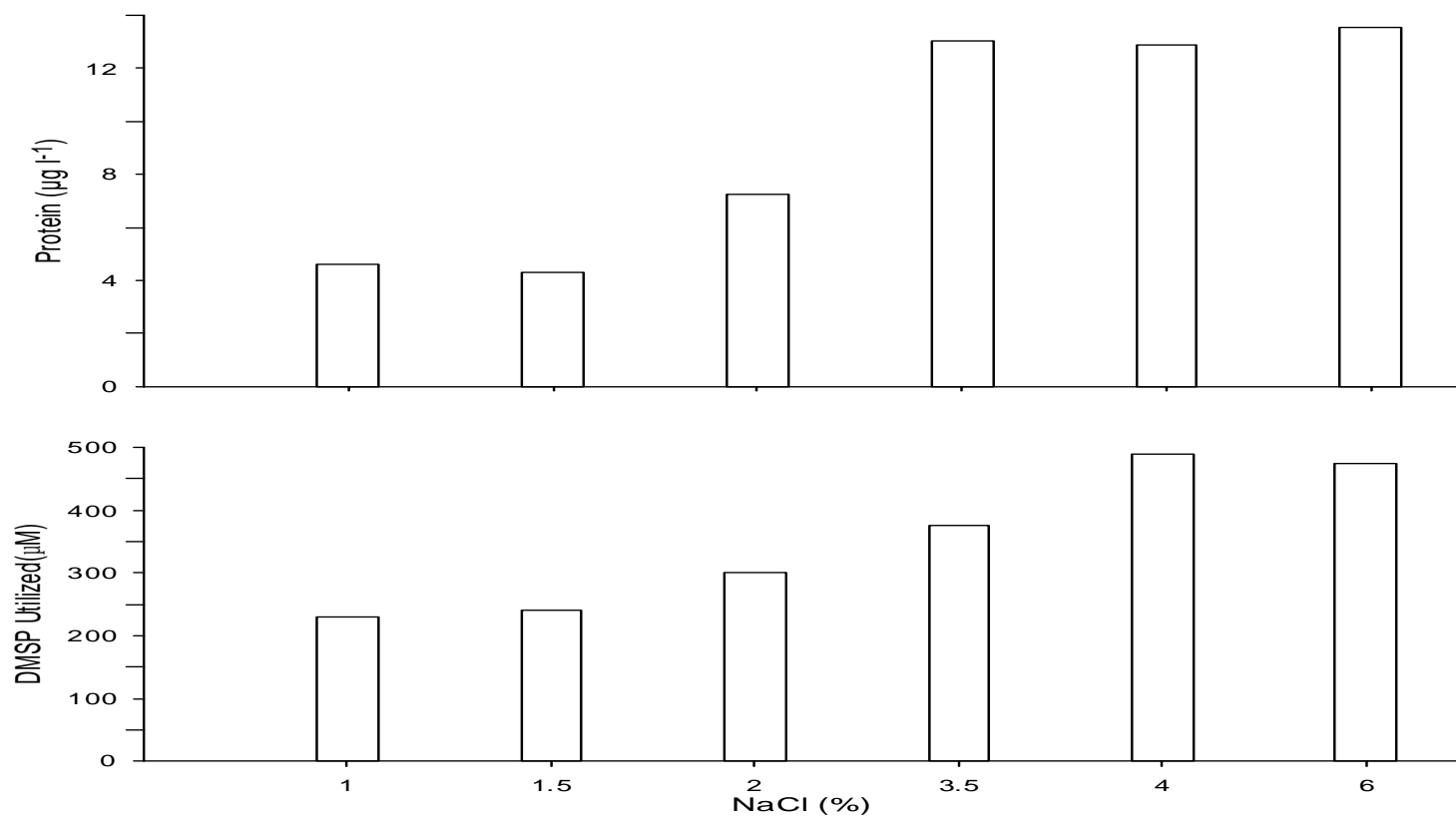


Fig. 43 The growth and DMSP utilization of strain *Bordetella petrii* (#1) at different NaCl concentrations

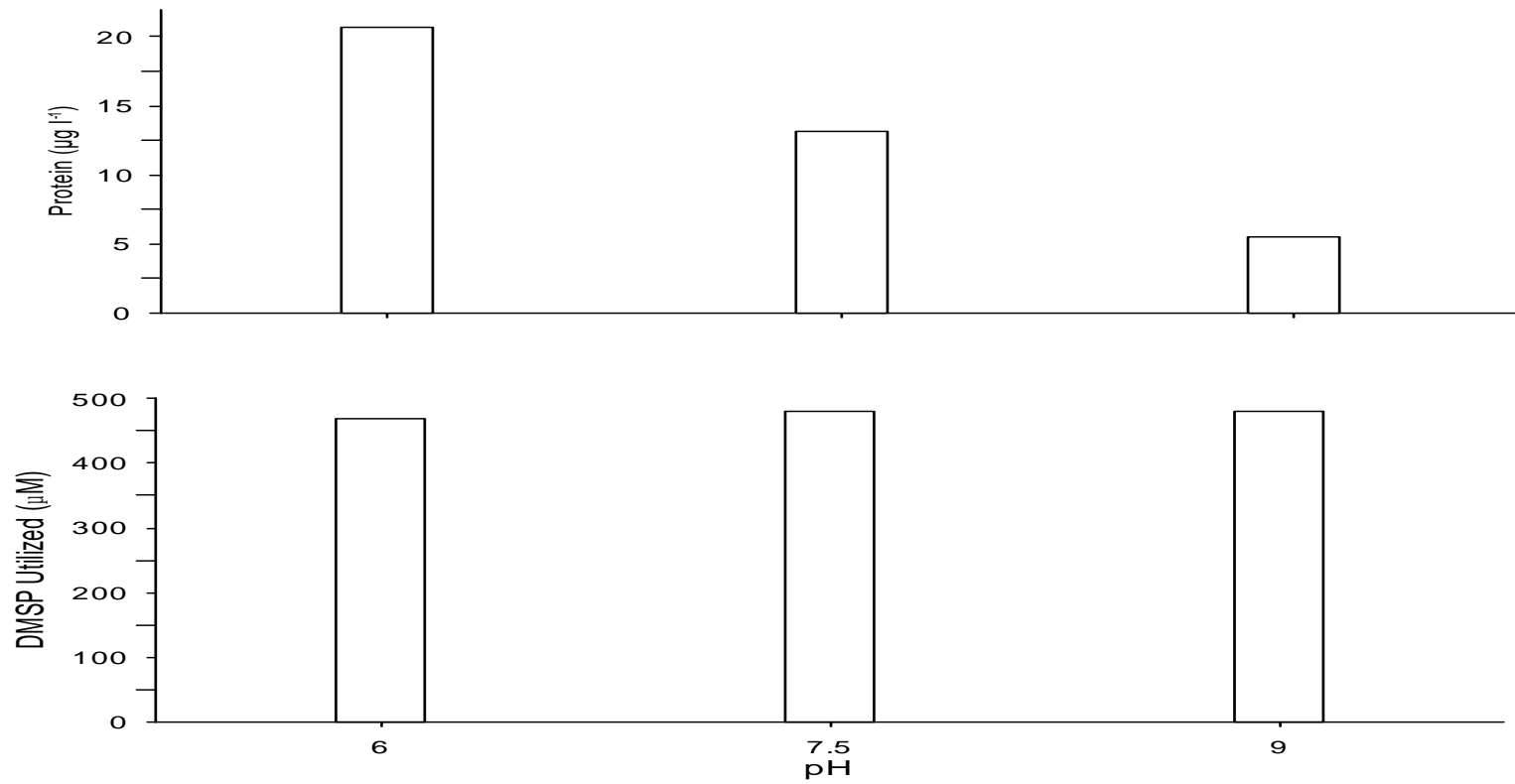


Fig. 44 The growth and DMSP utilization of strain *Bordetella petrii* (#1) at different pH

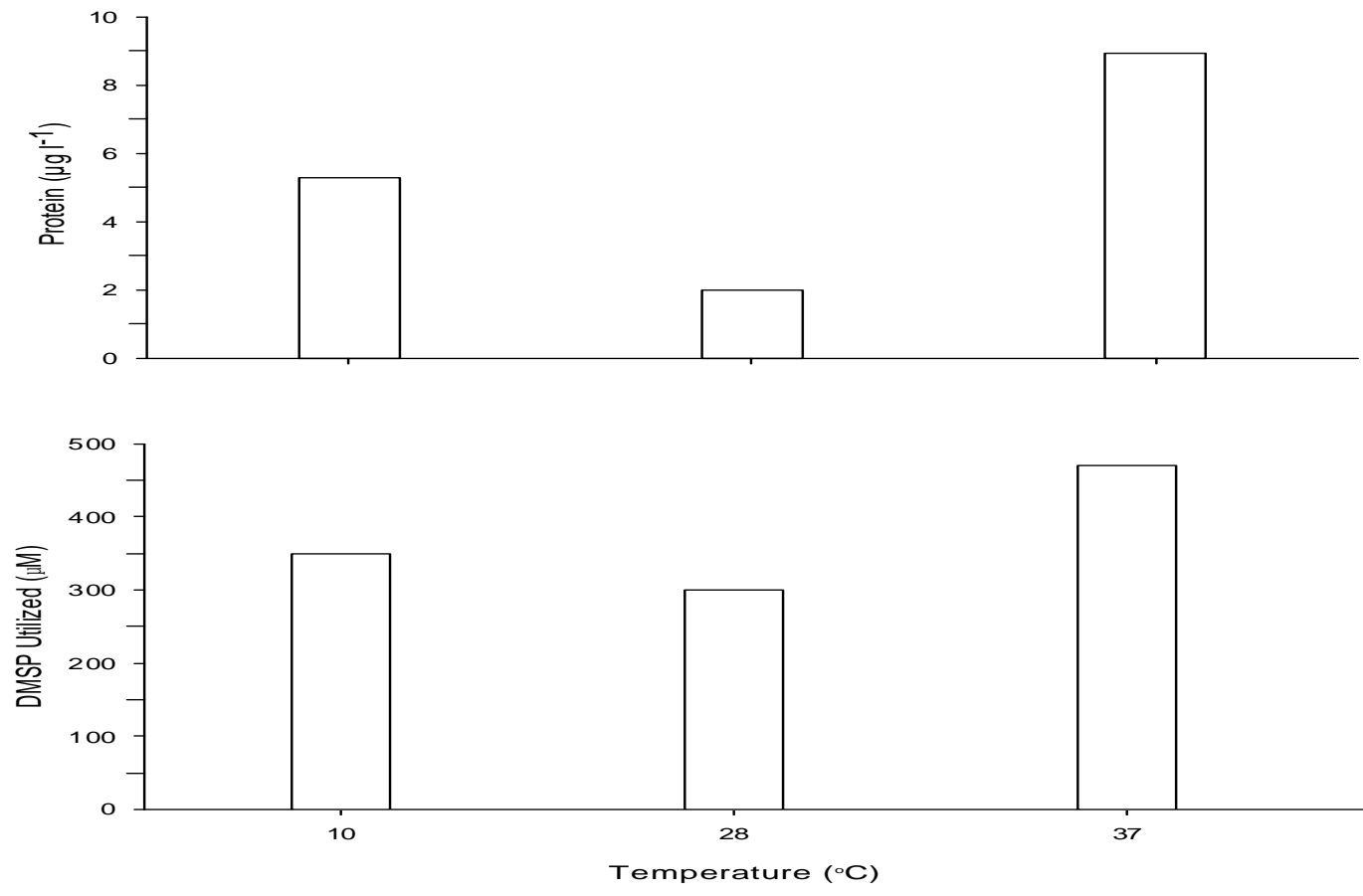


Fig. 45 The growth and DMSP utilization of strain *Bordetella petrii* (#1) at different temperatures

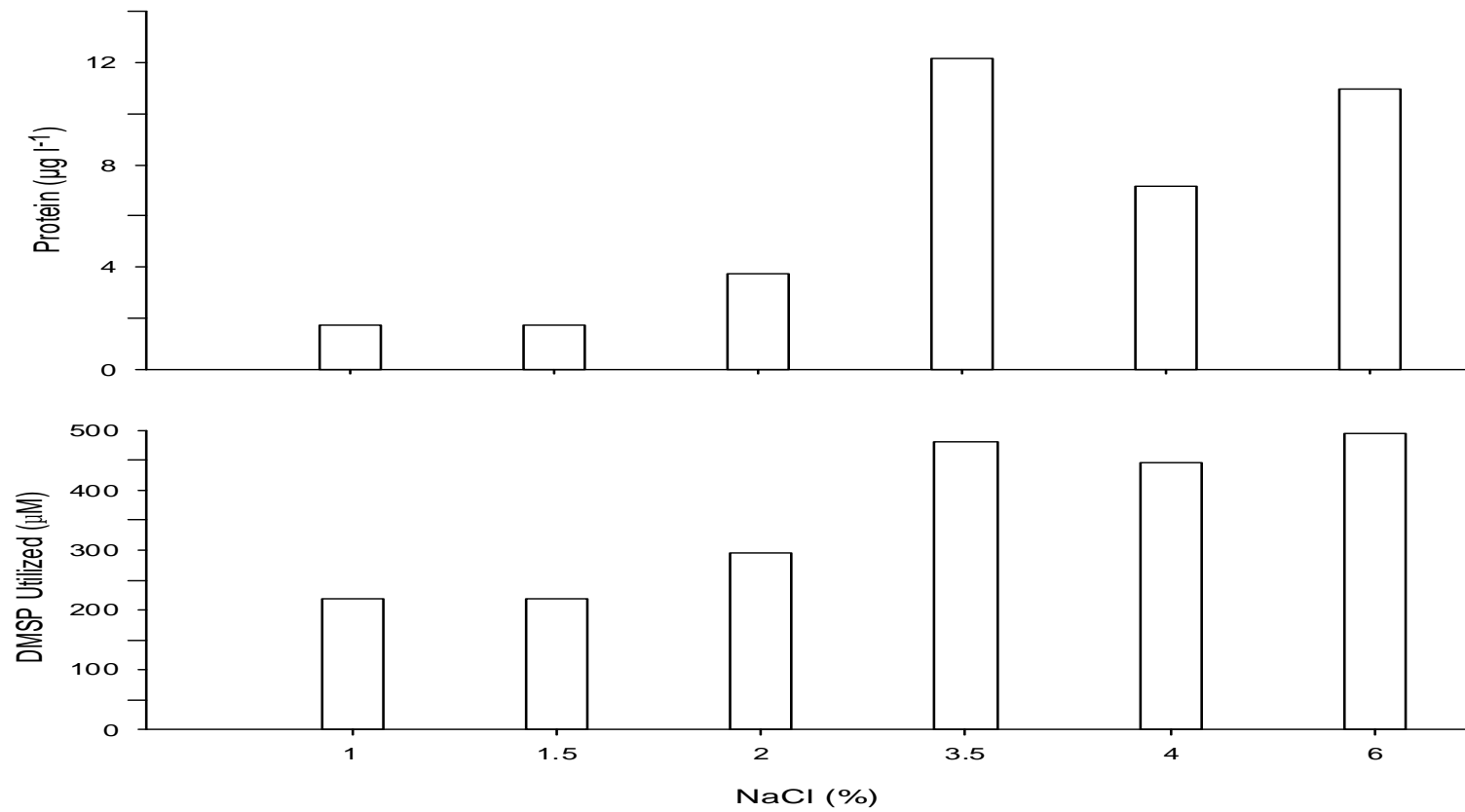


Fig. 46 The growth and DMSP utilization of strain *Bordetella petrii* (#2) at different NaCl concentrations

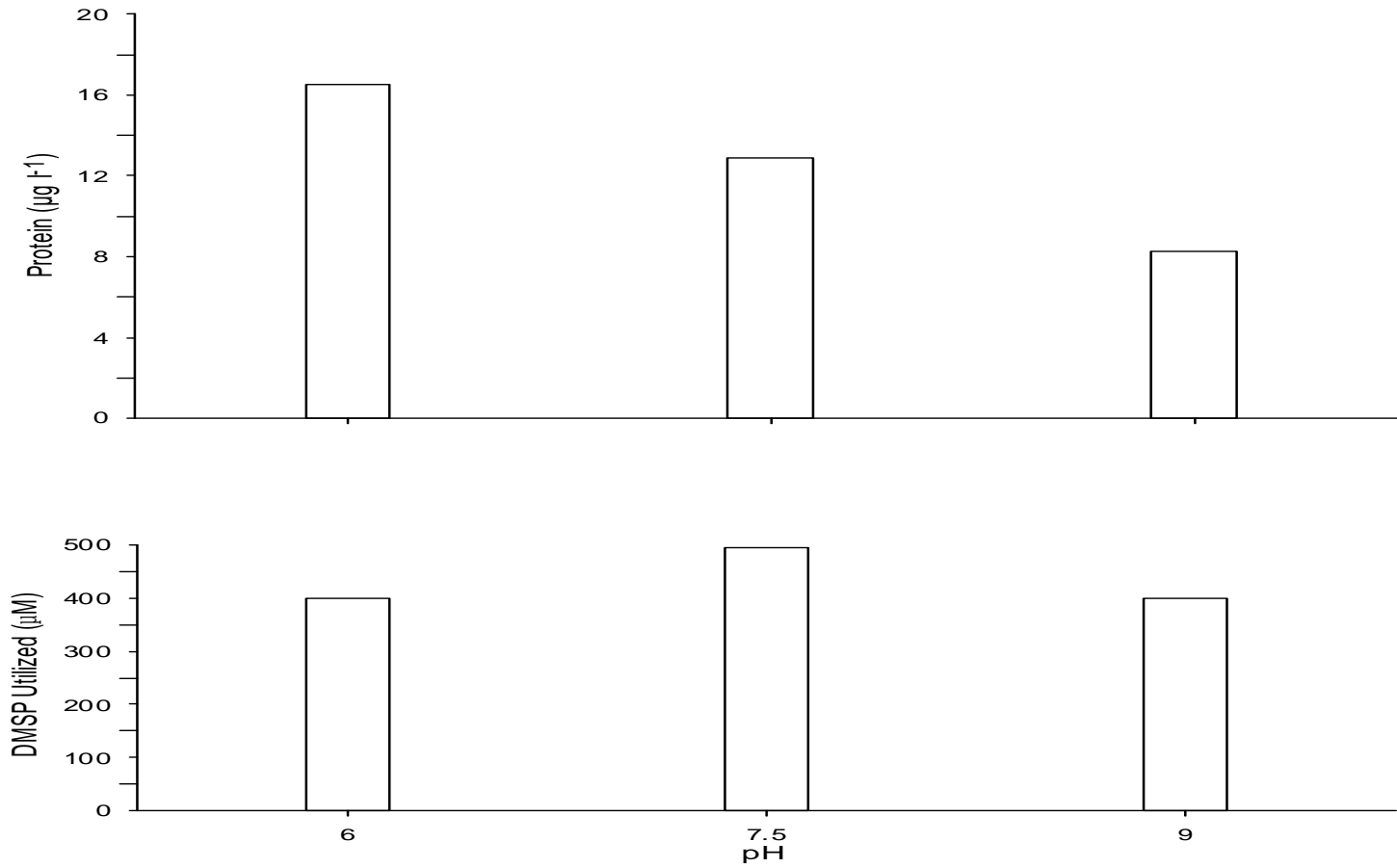


Fig. 47 The growth and DMSP utilization of strain *Bordetella petrii* (#2) at different pH

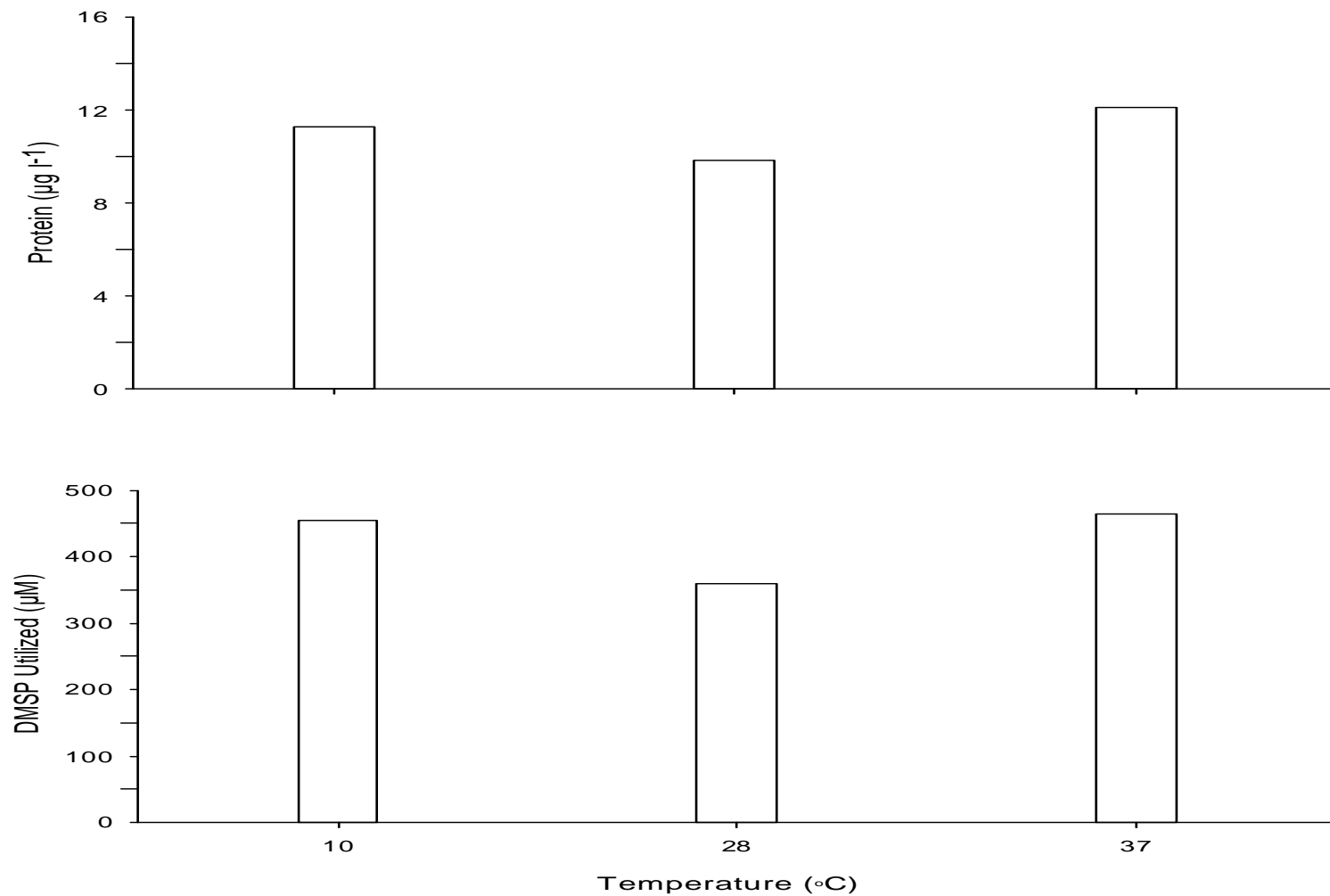


Fig. 48 The growth and DMSP utilization of strain *Bordetella petrii* (#2) at different temperatures

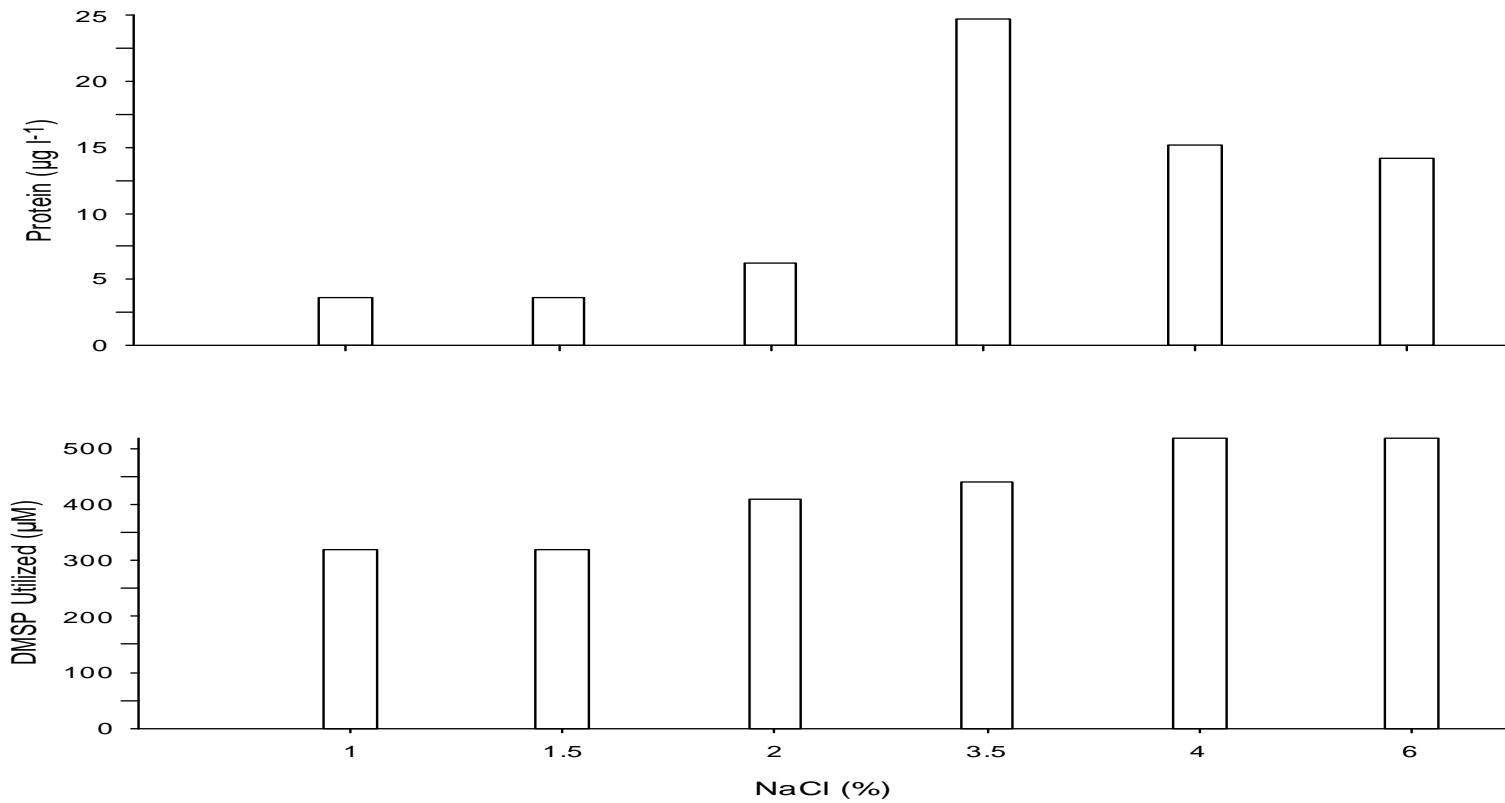


Fig. 49 The growth and DMSP utilization of strain *Bordetella petrii* (#3) at different NaCl concentrations

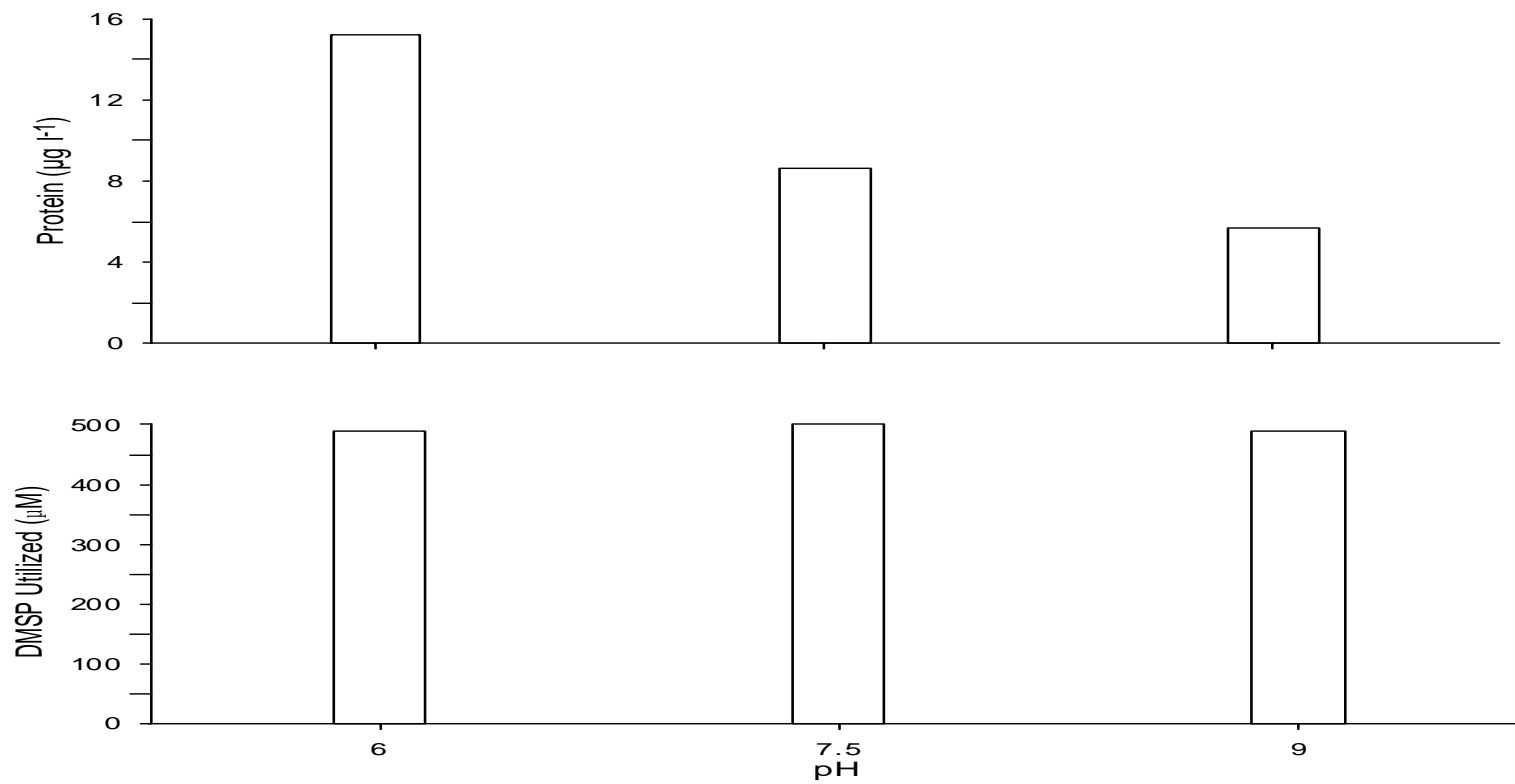


Fig. 50 The growth and DMSP utilization of strain *Bordetella petrii* (#3) at different pH

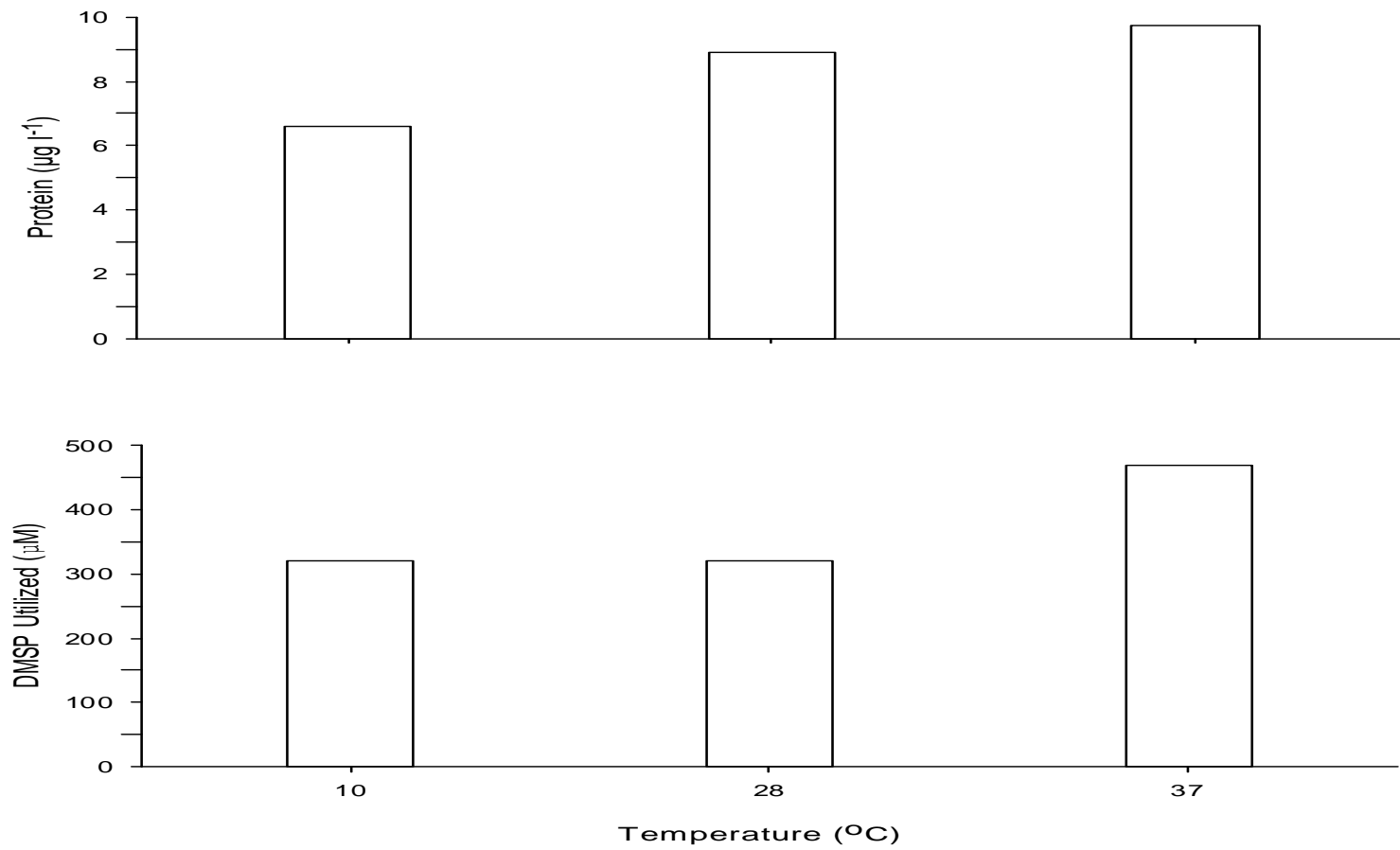


Fig. 51 The growth and DMSP utilization of strain *Bordetella petrii* (#3) at different temperatures

SI No	Substrates	<i>Alcanivorax dieselolei</i>
1	Tween 40	+
2	Tween 80	+
3	D-Arabitol	+
4	D-Galactose	+
5	Apha D Glucose	+
6	D-Mannose	+
7	D- Psicose	+
8	L-Rhamnose	+
9	Pyruvic Acid Methyl Ester	+
10	Cis Aconitic Acid	+
11	Cirtic Acid	+
12	D-Galactonic Acid Lactone	+
13	D-Gluconic Acid	+
14	Alpha Hydroxybutyric Acid	+
15	Beta Hydroxybutyric Acid	+
16	Gamma Hydroxybutyric Acid	+
17	Itaconuc Acid	+
18	Alpha Keto Butyric Acid	+
19	Alpha Keto Glutaric Acid	+
20	Apha Keto Valeric Acid	+
21	D,L,Lactic Acid	+
22	Propionic Acid	+

Table. 27 Carbon utilization pattern of the strain

Fatty Acids	<i>Alcanivorax dieselolei</i> (%)
C _{10:0}	0.68
C _{11:0} iso	3.32
C _{11:0} anteiso	0.09
C _{10:0} 3OH	0.16
C _{unknown 11.799}	1.03
C _{12 : 0}	0.74
C _{11:0} iso 3OH	1.78
C _{12 : 0} 2OH	0.58
C _{12 : 0} 3OH	2.85
C _{12:0} iso 3OH	0.41
C _{14 : 0}	2.31
C _{14:0} iso	0.58
C _{13:0} iso 3OH	2.9
C _{13:0} 2OH	1
C _{15 : 0}	0.54
C _{15:1} isoF	0.91
C _{15:0} iso	30.53
C _{15:0} anteiso	13.23
C _{16 : 0}	7.84
C _{16:0} iso	1.38
C _{16 : 1} ω 9C	2.75
C _{16:1} ω 7C/15 iso 2OH (Sum in Feature 3)	9.35
C _{iso 17:1} ω9c	5.34
C _{17:0} ISO	4.54
C _{17:0} ANTEISO	0.75
C _{17:1} ω 8c	0.33
C _{18:1} ω 9c	1.65
C _{18:1} ω 7c	1.21
C _{18:0}	0.37

Table. 28 Fatty acid profile of *Alcanivorax dieselolei*

Strain	Characteristics					
	Temperature for growth (°C)			NaCl for growth(%)		
	Minimal	Optimum	Maximum	Minimal	Optimum	Maximum
<i>Alcanivorax dieselolei</i>	18	28	40	0.5	3	7.5

Table. 29 a) Cell growth at different temperatures and NaCl concentrations.

Strain	Sugar	Cysteine	Acrylic acid
<i>Alcanivorax dieselolei</i>	+	-	-

Table. 29 b) Chemotactic response towards Sucrose, Cysteine and Acrylic acid.

Strain	pH 2			pH 6			pH 7.5		
	Conc of DMSP (percentage of responsive cells)								
	300µM	60nM	10pM	60µM	12nM	2pM	60µM	12nM	2pM
<i>Alcanivorax dieselolei</i>	18	60	100	10	68	100	18	3	0

Table. 29 c) Chemotactic response towards DMSP at different pH and concentrations.

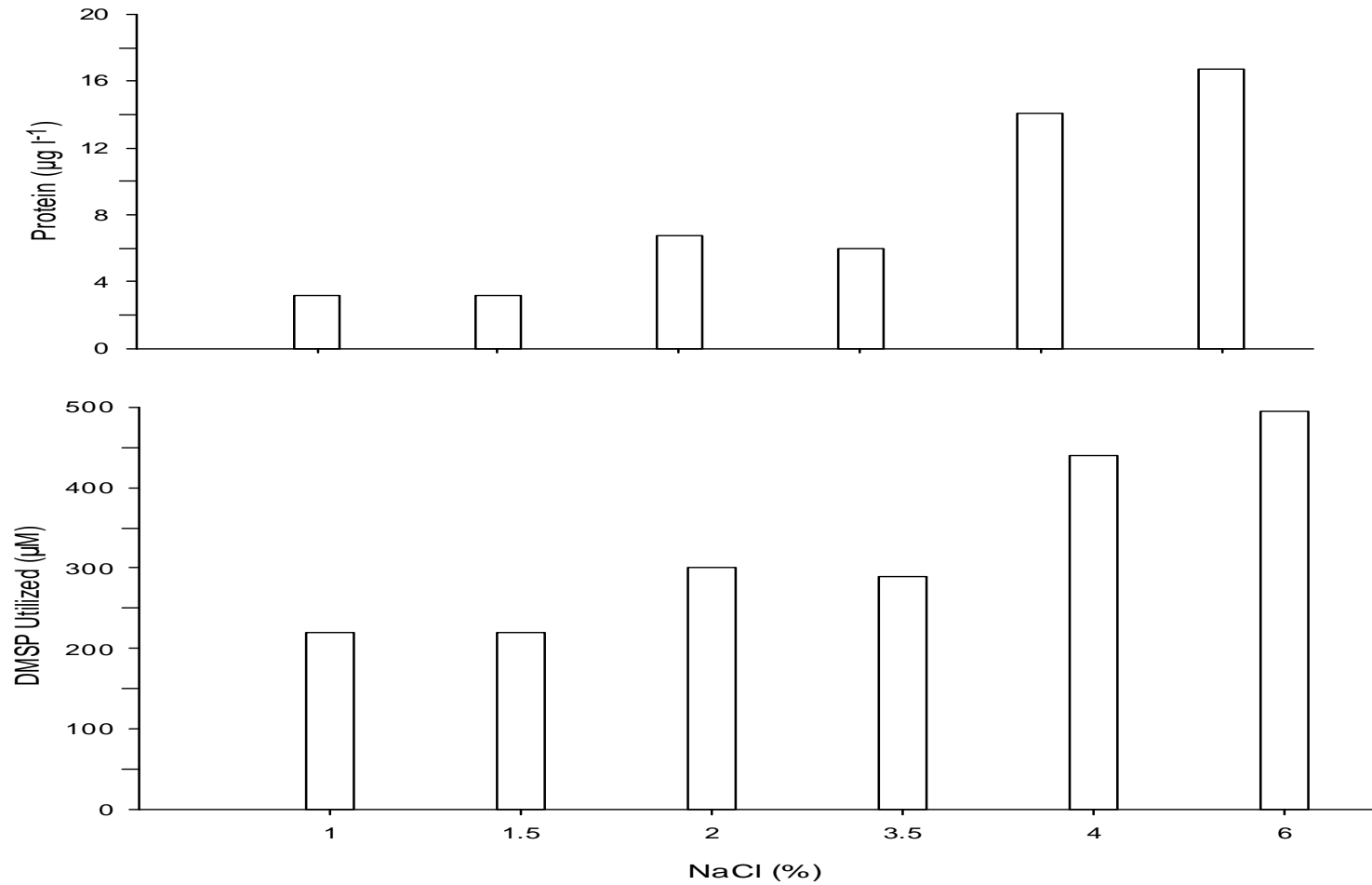


Fig. 52 The growth and DMSP utilization of strain *Alcanivorax dieselolei* at different NaCl concentrations.

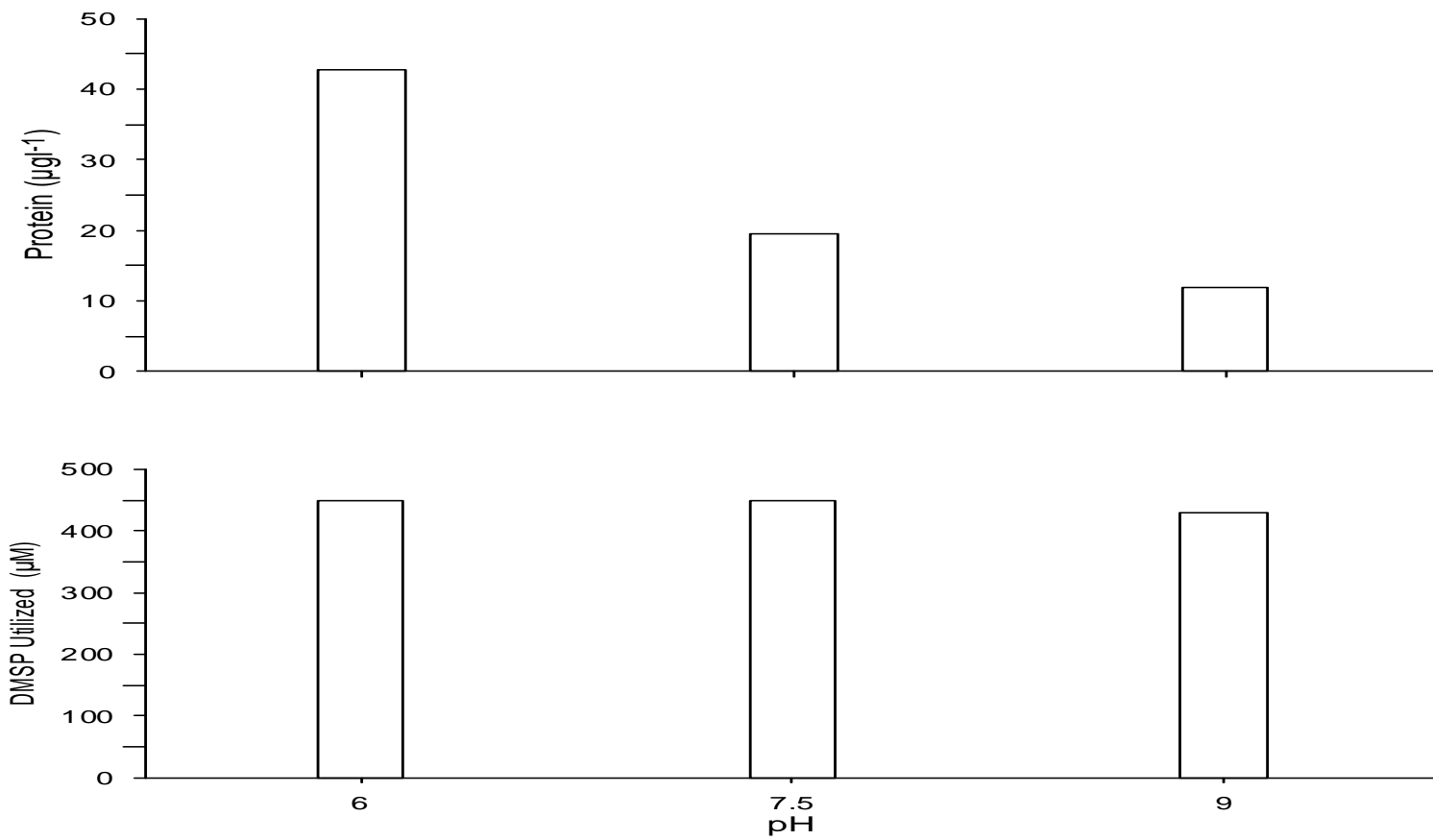


Fig. 53 The growth and DMSP utilization of strain *Alcanivorax dieselolei* at different pH.

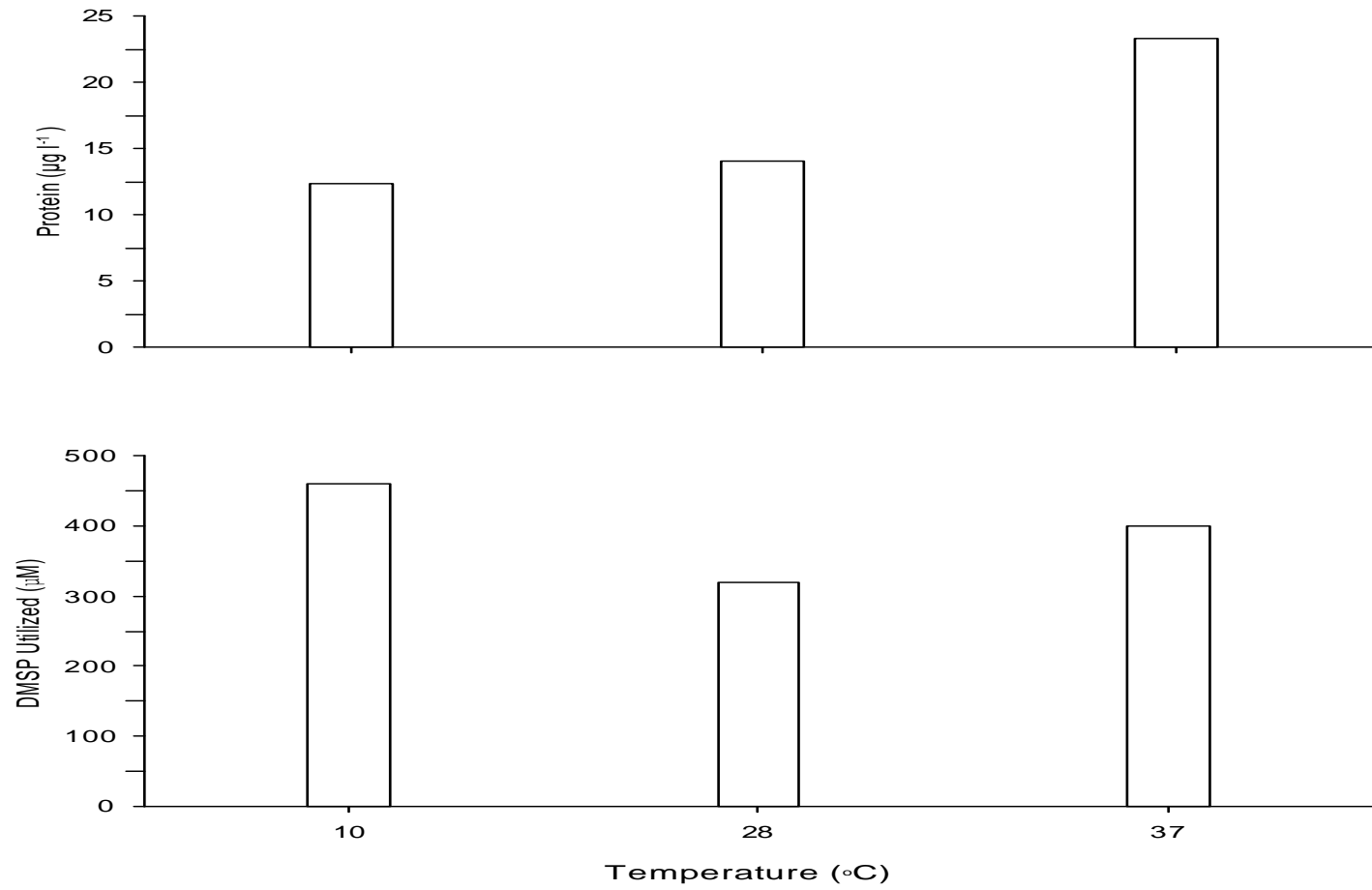


Fig. 54 The growth and DMSP utilization of strain *Alcanivorax dieselolei* at different temperatures.

Sl No	Substrates	<i>Bacillus sp</i>
1	N-Acetyl D Galactosamine	+
2	Adonitol	+
3	I-Erythritol	+
4	D-Fructose	+
5	Gentobiose	+
6	Beta Methyl D-Glucoside	+
7	L-Rhamnose	+
8	D-Sorbitol	+
9	D-Gluconic Acid	+
10	D,L,Lactic Acid	+
11	Propionic Acid	+
12	Quinic Acid	+
13	D-Saccharic Acid	+
14	Sebacic Acid	+
15	Succinic Acid	+
16	Glucuronamide	+
17	L Alanimide	+
18	D- Alanine	+
19	L-Alanine	+
20	L Asparagine	+
21	L-Glutsmic Acid	+
22	Glycyl L- Glutamic Acid	+
23	L Phenylalanine	+

24	L Proline	+
25	D Serine	+
26	L Serine	+
27	Inosine	+

Table. 30 Carbon utilization pattern of the strain

Fatty Acids	<i>Bacillus sp</i> (%)
C ₁₂ :0	0.22
C ₁₂ :0 2OH	0.09
C ₁₂ :0 3OH	0.2
C ₁₄ :0	5.66
C ₁₄ :0 2OH	2.17
C ₁₄ :1 ω 5C	0.16
C ₁₄ :0 3 OH/16:1 ISO 1 (Sum in Feature 2)	6.22
C ₁₆ :0	33.71
C ₁₆ :1 ω 7C/15 iso 2OH (Sum in feature3)	28.62
C ₁₆ :0 3OH	0.41
C _{iso} 17:0	0.41
C ₁₇ :0CYCLO	7.11
C ₁₈ :1 w 9c	0.55
C ₁₈ :1 w 7c	9.77
C ₁₈ :0	4.29

Table. 31 Fatty acid profile of the strain

Strain	Characteristics					
	Temperature for growth (°C)			NaCl for growth(%)		
	Minimal	Optimum	Maximum	Minimal	Optimum	Maximum
<i>Bacillus sp</i>	20	32	42	0.5	3	7.5

Table. 32 a) Cell growth at different temperatures and NaCl concentrations.

Strain	Sugar	Cysteine	Acrylic acid
<i>Bacillus sp</i>	+	+	-

Table. 32 b) Chemotactic response towards Sucrose, Cysteine and Acrylic acid.

Strain	pH 2			pH 6			pH 7.5		
	Conc of DMSP(percentage of responsive cells)								
	300µM	60nM	10pM	60µM	12nM	2pM	60µM	12nM	2pM
<i>Bacillus sp</i>	31	88	0	93	67	40	62	55	27

Table. 32 c) Chemotactic response towards DMSP at different pH and concentrations

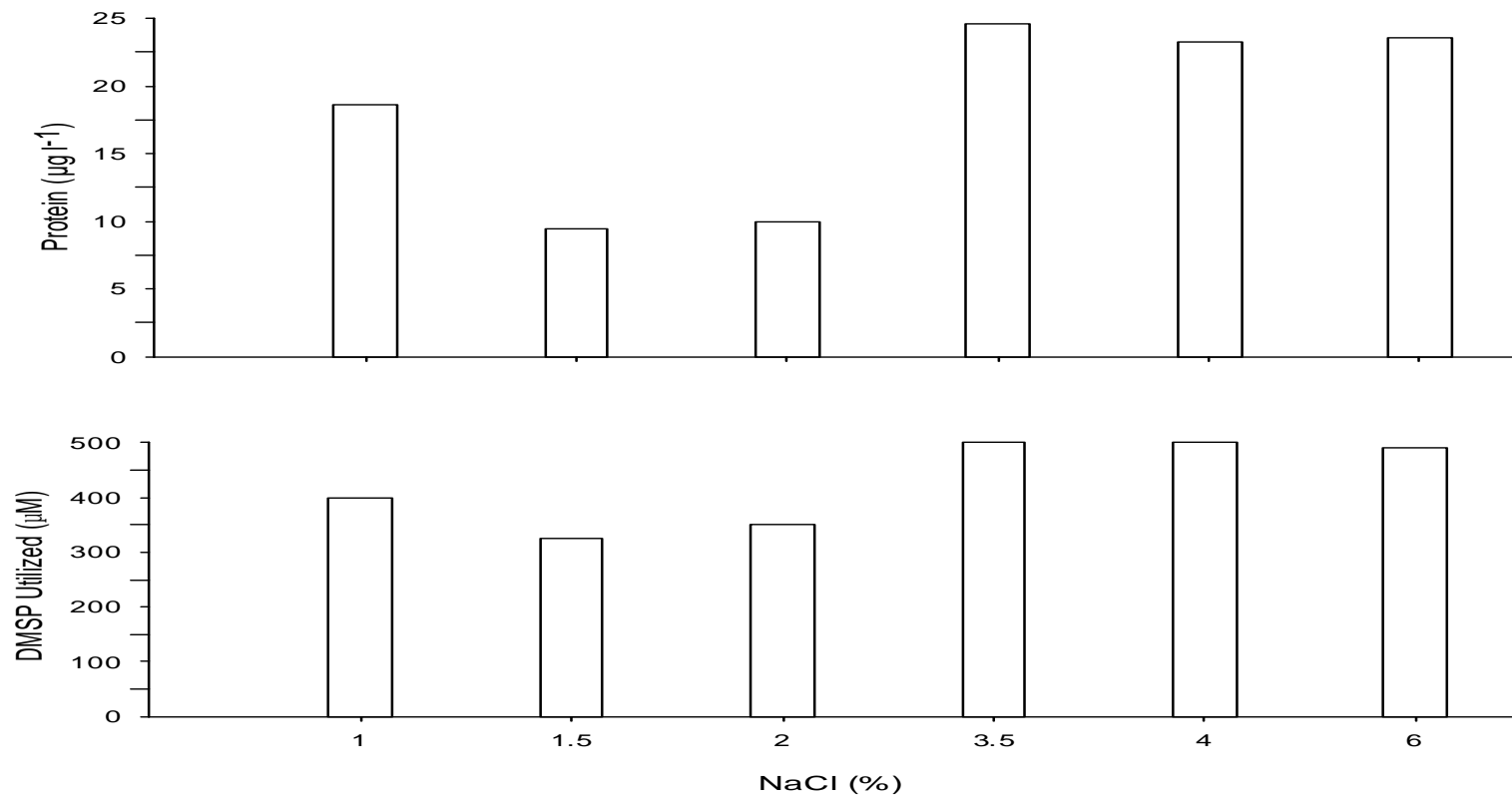


Fig. 55 The growth and DMSP utilization of strain *Bacillus sp* at different NaCl concentrations

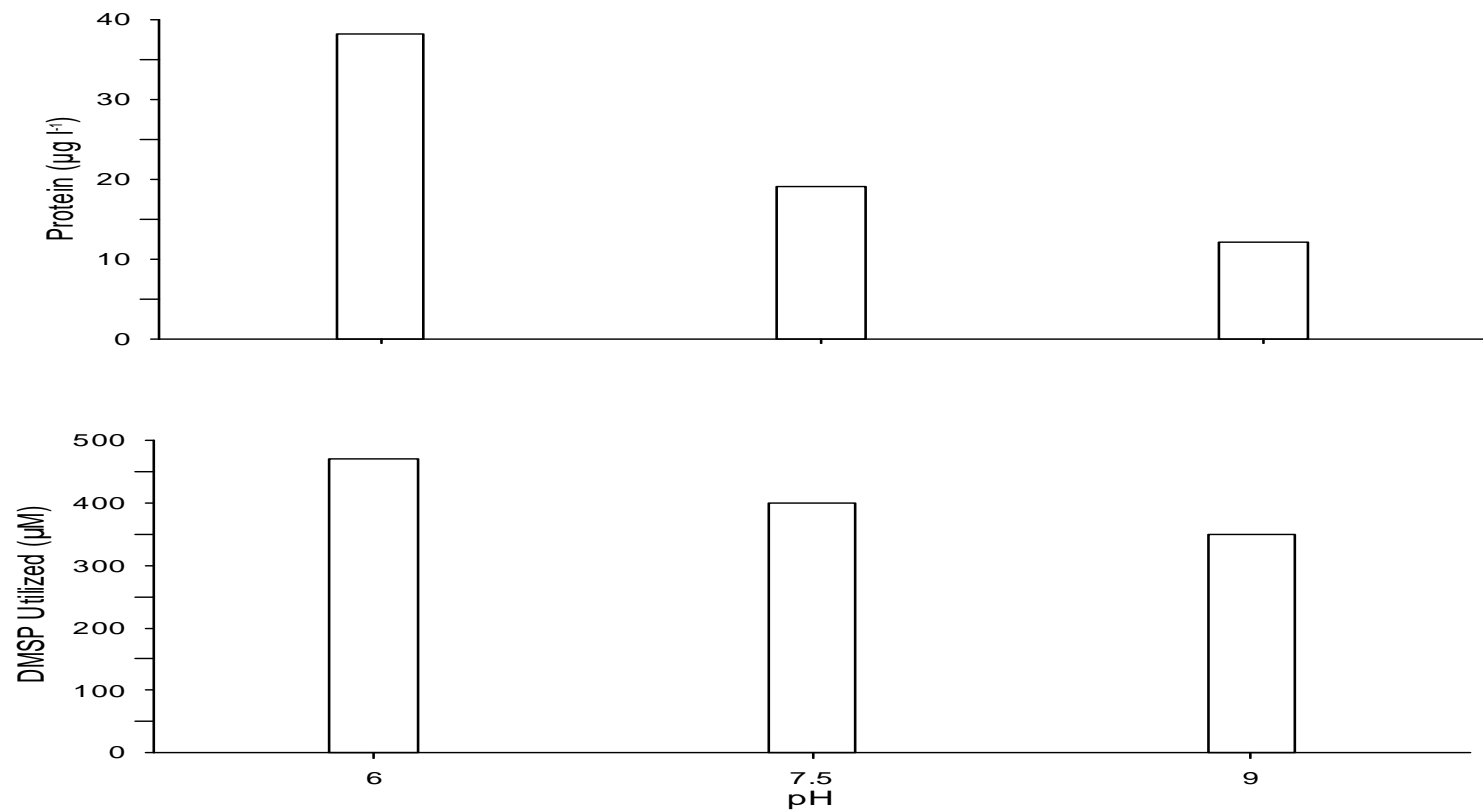


Fig. 56 The growth and DMSP utilization of strain *Bacillus sp* at different pH

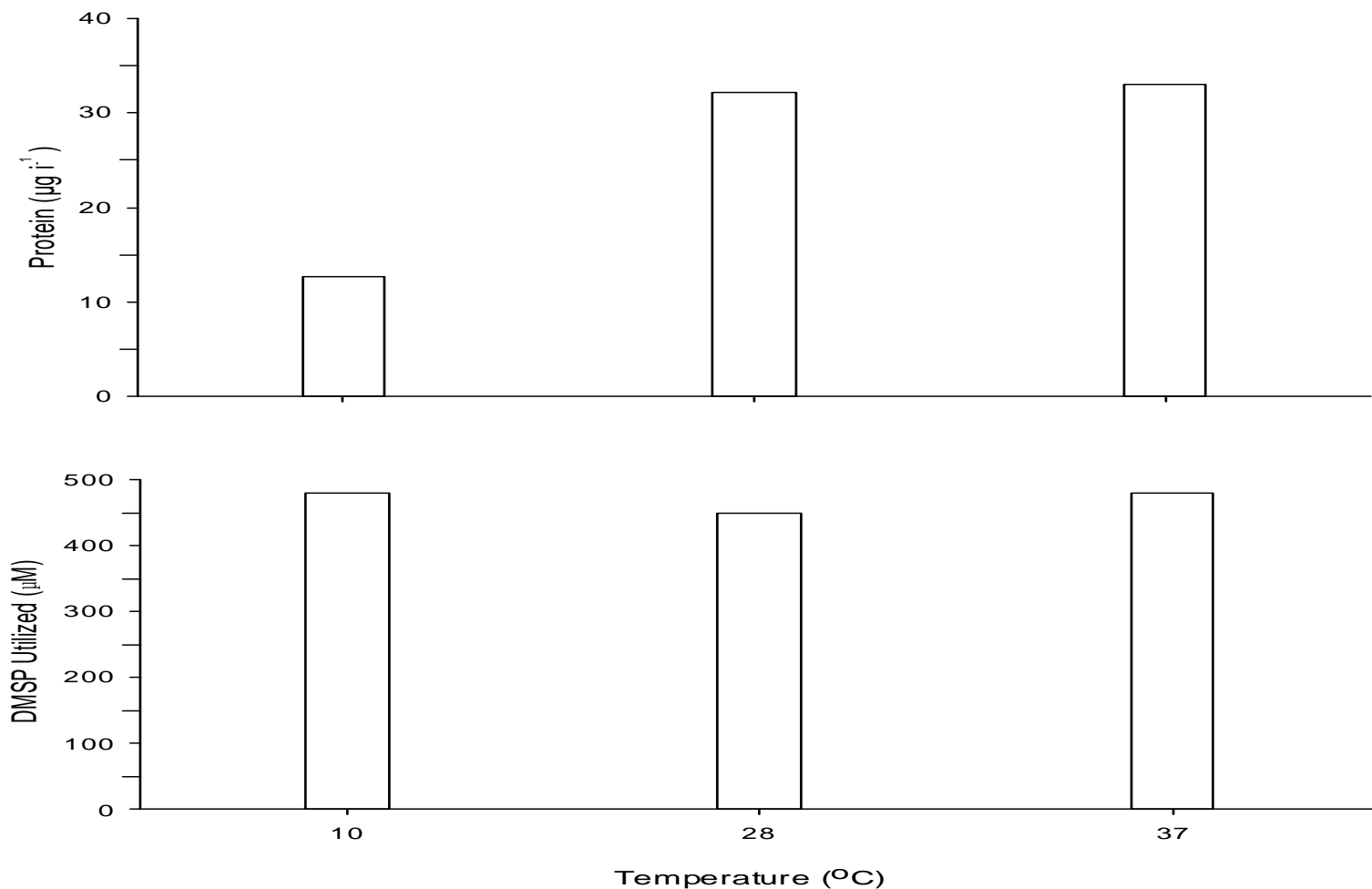


Fig. 57 The growth and DMSP utilization of strain *Bacillus sp* at different temperatures