

CHAPTER 2

Chapter 2A

2A.1 Introduction

Exopolysaccharides (EPS) are high molecular weight compounds and are sticky in nature (Sieburth et al 1976, Alldredge and Silver 1988, Alldredge et al 1993, Passow et al 1994). In the aquatic environments, phytoplankton blooms are one of the major sources of EPS with as much as 80% of fixed carbon being released into the surrounding waters (Myklestad and Haug 1972, Myklestad 1977, Myklestad et al. 1989). EPS is also derived from other sources like bacterial growth (25% of respired carbon), sloppy zooplankton feeding and cell-lysis etc. (Decho 1990, Kaltenböck and Herndl 1992, Stoderegger and Herndl 1998, Jacobsen 2000, Dilling and Alldredge 2000). In coastal waters, benthic macroalgae, microphytobenthos and land-derived organic matter may also act as additional sources of EPS.

The production and chemical characteristics of EPS are controlled by nutrient dynamics, microbial physiology, phytoplankton species, age of phytoplankton bloom etc. Similarly, EPS produced by microorganisms may exist either as tight capsules or as loosely attached slimes (Decho 1990, Bhosle et al 1995, Underwood et al 1995) that generally differ in their physico-chemical characteristics (Decho 1990, Hoagland et al 1993). For example, capsular EPS are less degradable and may persist for a longer period in the water column than slime EPS (Stoderegger and Herndl 1998). On the other hand, slimes play an important role in flocculation and mass sedimentation of

phytoplankton blooms (Kjørboe and Hansen 1993). Such differences in their chemistry and properties may lead to varying behavior of EPS in time and space.

EPS may directly or indirectly contribute to particulate (Passow et al 1994, Baldi et al 1997), colloidal (Sharp 1973, Hung et al 2001) or dissolved organic matter (Burney et al. 1979, Benner et al 1992, Borch and Kirchmann 1997, Myklestad et al 1997). Apart from several important functions including cell attachment, biofilm development, sequestering nutrients etc. (Decho 1990, Wingender et al 1999), EPS is also involved in stabilization of sediments (Decho 2000), organic carbon transformations through the microbial loop (Cho and Azam 1988, Decho and Herndl 1995) and metal cycling (Passow 2002) in the natural environments. EPS may also serve as an alternate carbon source for marine bacterioplankton (Fajon et al 1999, Goto et al 1999).

These polymers may coagulate spontaneously to form discrete exopolymeric particles called transparent exopolymeric particles (TEP) by biotic and/or abiotic processes (Alldredge et al 1993, Mopper et al 1995, Chin et al 1998, Zhou et al 1998). TEP are generally abundant during the decline of a bloom (Alldredge et al 1993, Passow and Alldredge 1994, Mari and Kjørboe 1996). Due to their global distribution, EPS may be considered the second largest organic carbon pool after dissolved organic carbon and contributes directly to the cycling of organic carbon (Benner et al 1992, Kepkay 1994, Amon and Benner 1994, Mari 1999).

Despite the numerous functions ascribed to EPS and TEP in aquatic environment, there is little information on their dynamics in near-shore, especially tropical waters. Most of the reports on the dynamics of EPS and/or TEP in near shore waters are either from temperate or polar waters (Kiørboe et al 1994, Schuster and Herndl 1995, Riebesell et al 1995, Hong et al 1997, Moreira and Martin 1998). In view of the above, the work presented in this chapter was planned to 1) assess the temporal variations of bacterial abundance, EPS (NaCl-soluble EPS and EDTA-soluble EPS) and TEP concentrations in the suspended particulate matter and sinking macroaggregates, 2) evaluate the influence of these polysaccharides on bacterioplankton abundance and 3) assess the contribution of these polysaccharides to the POC. These parameters along with hydrography and nutrients were monitored over a period of 26 months at a shallow water station in the Dona Paula Bay.

2A.2 Materials and Methods

2A.2.1 Sampling site

The sampling site was the Dona Paula jetty (15°27'N; 73°48'E), which had an average depth of 3m (**Fig. 2A.1**). The area experiences three seasons viz.: (1) southwest monsoon (June-September) when the area is inundated with riverine water and land runoff due to heavy precipitation. This brings about major changes in temperature, salinity, suspended load, dissolved oxygen, nutrients and organic matter composition. The water column near the study site experiences stratification due to fresh water influx during monsoon; (2) a

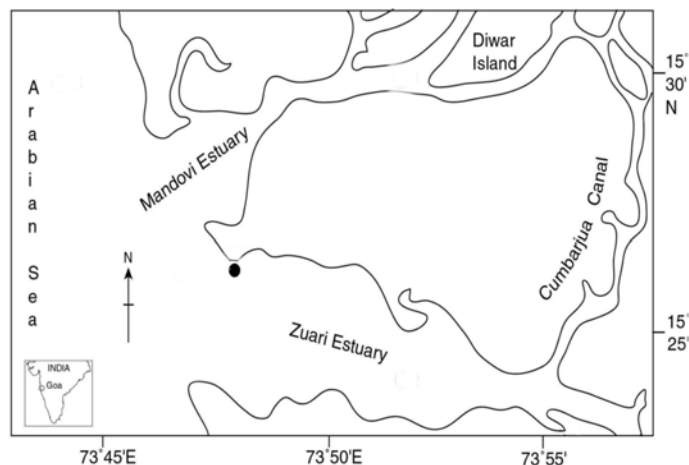


Fig. 2.1 The location map of the study site.

post monsoon (October-January) season which is a recovery period from freshwater domination with increase in salinity but little changes in surface water temperature and (3) a premonsoon season (February-May) when the water becomes typically marine (Qasim and SenGupta 1981, Gauns 2000). Earlier studies at this study site showed that the surface water temperature varied from 25°C to 33°C, whereas the surface salinity ranged from a low of 10 psu (during monsoon) to a high of 35 psu (pre-monsoon). Moreover, the study site is close to the mouth of a tidal estuary and hence experiences tidal currents ranging from 1 cm s⁻¹ in the post-monsoon to 25 cm s⁻¹ during late pre-monsoon months (Jayakumar, personal communication). The tidal range in the study area varied from 0.25 m (neap tide) to 2 m (spring tide) (Chandramohan et al 1998). Since the sampling site was close to the shore, it experiences heavy wave action during monsoon. The resuspension of bottom sediment is high during low tides and monsoon months. The mean significant wave height reported in these waters was 0.93 ± 0.18 m and the frequency of maximum height waves was 10 s (Vethamony and Kumar 1997). Nitrate

levels at the site vary from 1 μM in the pre-monsoon to 14 μM during monsoon (Devassy and Goes 1988). The site receives terrestrial organic inputs from the Zuari and Mandovi estuaries, which are fringed with thick mangrove coverage (Wafar et al 1997). The phytoplankton or micro-algal population is one of the major sources of organic carbon in the Dona Paula Bay with periodic blooms of diatoms like *Skeletonema*, *Nitzschia*, *Navicula*, *Coscinodiscus* and *Chaetoceros* during the months of September-October, December-January and April (Devassy and Goes 1988, Gauns 2000). Beds of macroalgae like *Sargassum* appear near the study site during the post-monsoon months. Moreover, the study site is a tourist spot and therefore experiences intense human activities.

Sampling

Surface seawater samples were collected using an acid clean plastic carboy during high tide at fortnightly intervals during 1998-99 and thereafter once a month in 1999-2000 for the analyses of various parameters. Macroaggregate samples were collected with a slight modification of the method of Grossart et al (1998). Briefly, a 5 l Niskin water sampler whose lower lid was kept closed and suspended at 1m below the surface. After 30 mins, the upper lid of the Niskin sampler was closed using a messenger and the sampler was retrieved. The sampler was brought to the laboratory and kept in an upright position for one hour to allow the macroaggregates to settle. The supernatant was then siphoned off and the content of the sampler was adjusted to a known volume. Both surface seawater and macroaggregate samples were prefiltered through

20 µm nylon mesh to remove large swimmers before filtering for different parameters.

Aliquots of seawater or macroaggregates were filtered onto preweighed ashed GF/F filter papers for various particulate parameters like dry weight, chlorophyll *a* (Chl *a*), particulate organic carbon (POC) and EPS (NaCl-soluble EPS and EDTA-soluble EPS). All the particulate samples except Chl *a* samples were oven dried at <45°C.

TEP samples were collected as described by Alldredge et al (1993) except that 0.22µm pore-sized Nuclepore polycarbonate membrane filters were used instead of 0.45µm pore-sized filters. Twenty five to fifty ml of seawater or macroaggregate samples for quantitative estimation of TEP and 1 or 2 ml for microscopic enumeration and size estimation of TEP were filtered onto 0.22µm Nuclepore polycarbonate membrane filters using <10 mbar pressure. The sampling was carried out in duplicates.

Sub-samples of seawater and macroaggregates were fixed with formalin (4% final concentration) for the enumeration of total bacteria (Parsons et al 1984).

2A.2.2 Wind speed, hydrography and nutrients

Surface wind speed data were obtained from the local meteorological station (Meteorological Station, Altinho-Panjim). Sea surface temperature was recorded *in situ* using a thermometer. Seawater samples were brought to the

laboratory within half an hour of collection and analyzed for salinity, dissolved oxygen and nutrients (nitrate & phosphate). Salinity was estimated following the silver nitrate titration method (Stickland and Parsons 1975). Samples for dissolved oxygen were fixed and analyzed following the Winkler's method (Parsons et al 1984). Dissolved nitrate and phosphate were estimated spectrophotometrically (Parsons et al 1984). All samples were analyzed in duplicates.

2A.2.3 Suspended particulate matter and Macroaggregates

Dry weight

Filter papers with suspended particulate matter (suspended load) or macroaggregate (total macroaggregates) were washed with distilled water, oven dried at ~45°C and weighed on a microbalance (Model Mettler Toledo-AT 20) to estimate the dry weight.

Chlorophyll a (Chl a)

The Chl a samples were extracted overnight in 90% acetone. After extraction, the samples were centrifuged and the supernatant was measured for its fluorescence at 430nm excitation and 670nm emission wavelengths using a fluorescence spectrophotometer (Hitachi model F-2000). The method was calibrated using standard chlorophyll a.

Particulate organic carbon (POC)

The POC samples were analyzed following the dichromate oxidation method (Parsons et al 1984). Briefly, the filter paper containing particulate matter was taken in acid-cleaned test-tube and treated with 1 ml phosphoric acid. The samples were heated in a boiling water bath for 30 min. Thereafter, sulphuric acid-dichromate mixture was added and the tubes were further heated in boiling water bath for 60 min. Samples were then diluted to a known volume using distilled water and cooled. Aliquots of the samples were centrifuged and the supernatant was measured at 440 nm. Plain filters were treated in the same fashion and used as blanks. Glucose was used as standard for the calibration of method.

Exopolysaccharides (EPS)

Suspended particulate matter or macroaggregate samples were treated with 1.5M NaCl or 10mM EDTA so as to extract NaCl-soluble EPS or EDTA-soluble EPS, respectively (Bhosle et al 1995, Underwood et al 1995). Samples were extracted for 15 minutes using vortex mixer and then centrifuged at 3000 rpm (2062 g) for 10 mins. To 1 ml of the supernatant, 1 ml of cold 5 % phenol solution followed by 5 ml concentrated sulphuric acid were added. Samples were cooled and measured spectrophotometrically at 490 nm (Dubois et al 1956). Plain filters were treated as above and used as blanks. Glucose was used as a standard for calibration. Results are expressed as glucose equivalent.

Bacterial abundance

Total bacterial abundance was estimated following the acridine orange staining method (Parsons et al 1984). Briefly, 100-200 µl of the acridine orange stain (0.1%) was added to duplicate sterile tubes containing 1-2ml of the formalin fixed samples and incubated in dark for 2 min. The samples were then filtered onto 0.22µm black Nuclepore polycarbonate membrane filters. The filters were then placed on a drop of fluorescence free oil, another drop of oil placed onto it and covered with a cover slip. The slide was then viewed under oil immersion lens using a blue filter with a UV lamp as a source of light. About 10-12 fields were counted per filter and the average field count was used to calculate the abundance of bacteria as follows:

$$\text{Bacterial abundance/ml: Mean counts} \times \frac{\text{filter area}}{\text{field area}} \times \frac{1}{\text{ml filtered}}$$

TEP- chemical quantification

In order to quantify TEP, filters containing the particulate matter samples were stained with prefiltered (0.22 µm) alcian blue (0.02% in 0.06% glacial acetic acid; pH=2.5) and filtered dry under low vacuum (Passow and Alldredge 1995b). Blanks were prepared by staining empty filters with alcian blue.

Both TEP and blanks were treated with 6 ml of 80% sulfuric acid for 2h with intermittent shaking. The samples were then centrifuged and the supernatant

was measured spectrophotometrically at 787 nm (Passow and Alldredge 1995b). The TEP concentration was estimated as follows:

$$\text{TEP} = (E_{787} - B_{787}) \times F_x V_f^{-1}$$

where E_{787} is sample extinction, B_{787} is blank extinction, F_x is the factor of calibration and V_f is the volume filtered in litres. The factor F_x was calculated from the equation

$$F_x = M[(E_{787} - B_{787})V_{st}^{-1}]^{-1};$$

where M is the weight of the standard ($\mu\text{g l}^{-1}$ Xanthan gum) and V_{st} is the volume of the standard filtered in liters. Xanthan gum was used as a standard for calibration. The coefficient of variance for the entire procedure was 35%. The TEP concentrations are expressed as Xanthan gum equivalent.

2A.2.4 Statistical Analyses

In order to assess the correlation between various parameters, correlation matrices were obtained for both suspended particulate matter and macroaggregates. As the parameters showed strong seasonal variations and behaved differently in the two years, the correlation matrix was calculated for each year separately. The statistical analyses were carried out using STATISTICA 5.0.

2A.3 Results

2A.3.1 Surface Winds

During the sampling period, the wind speed varied from a high of 4.8 m s^{-1} (July 2000) to a low of 1.9 m s^{-1} (October 1999) (**Fig. 2A.2a**) with an average wind speed of $2.5 \pm 0.77 \text{ m s}^{-1}$. Seasonally, the wind speed was higher ($> 3 \text{ m s}^{-1}$) during south-west monsoon (June-September), reduced with the onset of post monsoon (October- January) season and did not fluctuate much during rest of the sampling period.

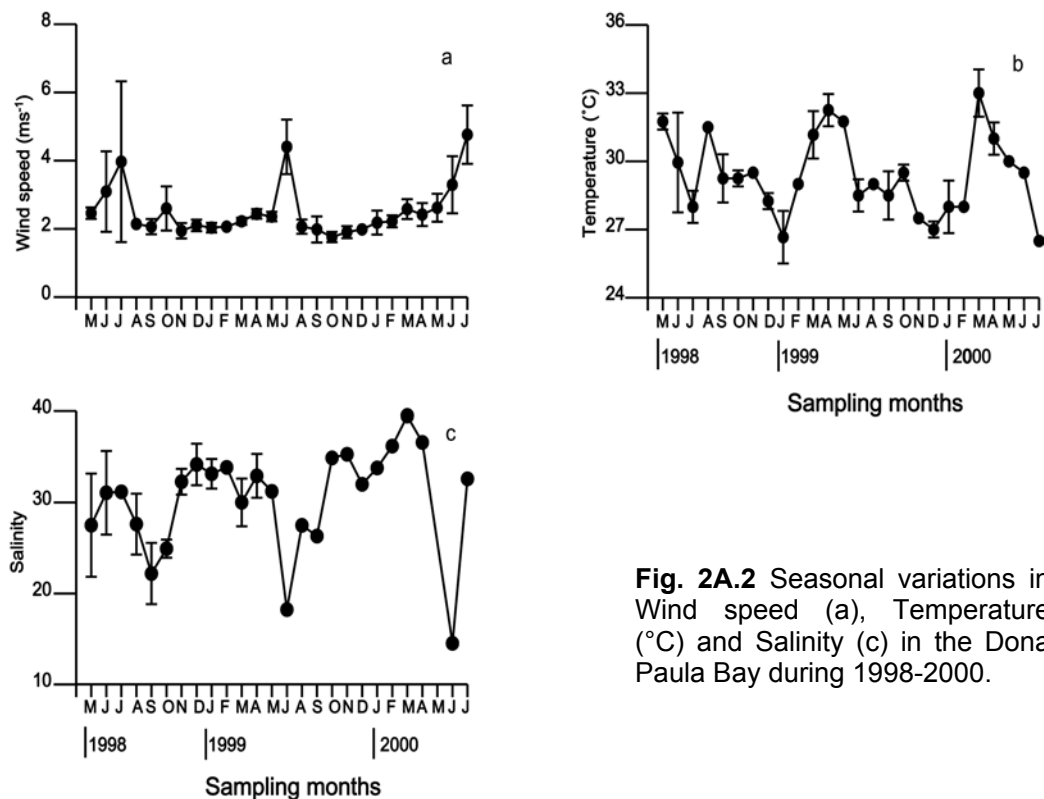


Fig. 2A.2 Seasonal variations in Wind speed (a), Temperature ($^{\circ}\text{C}$) and Salinity (c) in the Dona Paula Bay during 1998-2000.

2A.3.2 Hydrography

The surface water temperature at the study site showed large-scale variations and ranged from 26.5°C (July 2000) to 33°C (March 2000) (**Fig. 2A.2b**). The surface water temperature was lowest in the post-monsoon (October-January) (26°C to 28°C), moderate in monsoon (June-September) (28 to 30°C) and higher in the pre-monsoon (February-May) (31°C to 33°C) during the 26-month study period. The surface salinity for the entire study period varied from 14.7 (June 2000) to 39.7 (March 2000) (**Fig. 2A.2c**).

Over the period of sampling, the average dissolved oxygen concentration for the study period was $104.9 \pm 11.5 \mu\text{M}$. The seasonal variation in dissolved oxygen was conspicuous during the sampling period. The oxygen concentrations showed small variations between May '98 to March '99 and varied from $94 \pm 28.1 \mu\text{M}$ (August '98) to $120.6 \pm 5.8 \mu\text{M}$ (March 1999) (**Fig. 2A.3a**). Subsequently, the oxygen values declined continuously until November '99 ($78.8 \pm 2.3 \mu\text{M}$) except for a high in July '99 followed by a sharp increase in December 99 ($123 \mu\text{M}$). Thereafter the oxygen values did not vary much for the rest of the sampling period.

2A.3.3 Nutrients

The surface water nitrate concentrations displayed distinct seasonal rhythm and were influenced by the southwest monsoon (June-September). The nitrate concentration for the study period ranged from $0.03 \mu\text{M}$ (September '99) to $14.1 \mu\text{M}$ (July '99). The nitrate concentrations were higher in the monsoon (June-September) ($5.7 \pm 2.2 \mu\text{M}$) and declined in the post-monsoon (October-January) season ($2.5 \pm 0.4 \mu\text{M}$) (**Fig. 2A.3b**). Similarly, secondary maximal concentrations were observed in December '98 and March 2000.

Unlike the nitrate, the phosphate concentrations in the surface waters did not show any specific seasonal trend (**Fig. 2A.3c**). On the whole, the phosphate concentrations varied $0.09 \pm 0.03 \mu\text{M}$ (November '99) to $2.7 \pm 2.9 \mu\text{M}$ (April '99).

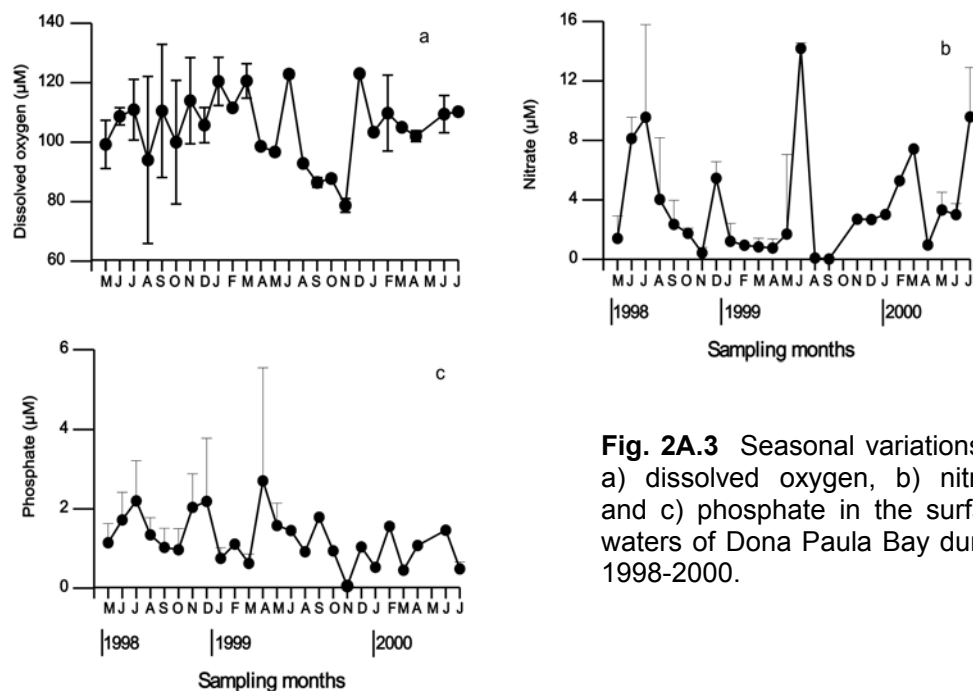


Fig. 2A.3 Seasonal variations of a) dissolved oxygen, b) nitrate and c) phosphate in the surface waters of Dona Paula Bay during 1998-2000.

2A.3.4 Dry weight

The dry weight of suspended particulate matter (suspended load) in the surface waters ranged from 3.4 (September '99) to 44.9 mg l⁻¹ (June '98) (**Fig. 2A.4a**). The suspended load was higher during monsoon (June-September) (21.3 mg l⁻¹) and lower in post-monsoon (October-January) (11.4 mg l⁻¹) followed by a small increase in pre-monsoon (February-May) (14.3 mg l⁻¹) (**Table 2A.3**).

The macroaggregates dry weight ranged from 2.9 (February '99) to 185.5 mg l⁻¹ (July '99) during the 26-month study and followed a seasonal pattern similar to that of suspended load (**Fig. 2A.4b**). The macroaggregate concentrations were higher in the monsoon (June-September) (45.7 ± 54.3 mg l⁻¹) and lower in post-monsoon (October-January) (20.7 ± 12.3 mg l⁻¹) (**Table 2A.4**).

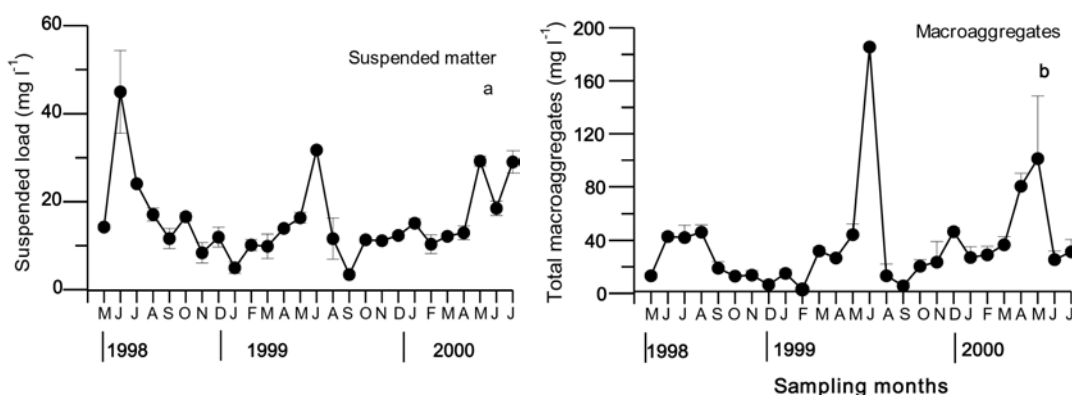


Fig. 2A.4 Seasonal variations in the concentrations of a) Suspended load and b) Total macroaggregates in the surface waters of Dona Paula Bay during 1998-2000.

Table 2A.1: Mean, SD, maximum and minimum range of various particulate parameters in the suspended matter during the period 1998-2000 in the Dona Paula Bay.

Parameters		Mean	SD	Max	Minimum
Suspended load	mg/l	15.9	9.2	45.0	3.4
POC	µg/l	700.5	256.1	1311.2	270.1
Chl <i>a</i>	µg/l	4.8	2.7	12.3	1.2
TBA	x 10 ⁶ cells/ml	2.4	2.0	8.2	0.5
NaCl-soluble EPS	µg/l	24.5	20.3	101.6	4.5
EDTA-soluble EPS	µg/l	64.4	41.0	210	26.2
TEP	µg Xan.eq./l	55.6	35.6	149.1	1.3
Phyto-C (%POC)		32.2	20.4	71.6	5.5
Bac-C (% POC)		4.7	4.8	20.8	0.5
NaCl-soluble EPS- C (% POC)		1.6	1.2	5.4	0.4
EDTA-soluble EPS- C (% POC)		4.0	2.0	11.2	1.4
TEP-C (% POC)		6.9	5.9	22.8	0.1

Chl *a*: Chlorophyll *a*; POC: Particulate organic carbon; TEP: Transparent exopolymer particles; TBA: Total Bacterial Abundance; Phyto-C: Phytoplankton carbon; Bac-C: Bacterial carbon.

Table 2A.2: Mean, SD, maximum and minimum range of various particulate parameters in the macroaggregates during the period 1998-2000 in the Dona Paula Bay.

Parameters		Mean	SD	Max	Minimum
Total					
macroaggregates	mg/l	36.3	37.6	185.5	2.9
POC	µg/l	1116.1	960.3	3326.6	156.6
Chl <i>a</i>	µg/l	2.7	1.7	6.2	0.4
TBA	x 10 ⁶ cells/ml	2.6	2.1	9.5	0.5
NaCl-soluble EPS	µg/l	24.0	21.7	84.5	0.9
EDTA-soluble EPS	µg/l	25.3	26.7	79.2	0.1
TEP	µg Xan eq/l	17.8	17.5	72.3	0.4
Phyto-C (%POC)		19.3	18.1	62.7	0.4
Bac-C (% POC)		4.6	4.8	19.9	0.1
NaCl-soluble EPS-C (% POC)		1.7	1.7	6.4	0.1
EDTA-soluble EPS-C (%POC)		2.7	2.3	7.9	0.3
TEP-C (% POC)		2.0	2.0	4.9	0.02

Chl *a*: Chlorophyll *a*; POC: Particulate organic carbon; TEP: Transparent exopolymer particles; TBA: Total Bacterial Abundance; Phyto-C: Phytoplankton carbon; Bac-C: Bacterial carbon.

Table 2A.3 : Average concentration of the various parameters in the suspended particulate matter in different seasons.

Parameters	Monsoon	Post-monsoon	Pre-monsoon
Suspended load ¹	21.3 ± 12.6	11.4 ± 3.6	14.3 ± 6.4
POC ²	760.1 ± 264.3	606.4 ± 182.2	708 ± 330
Chl <i>a</i> ²	3.8 ± 2.5	5.6 ± 3.1	5.3 ± 2.5
NaCl-soluble EPS ²	17.6 ± 6.9	33.8 ± 30.5	19.1 ± 12.5
EDTA-soluble EPS ²	69.6 ± 24.3	66 ± 59.5	59 ± 41
TEP ³	57.7 ± 44.6	50.1 ± 22.8	58.6 ± 38.6
TBA ⁴	1.7 ± 1.4	4 ± 2.4	1.5 ± 1.1
%Phyto-C	24.9 ± 22.2	36.8 ± 15.5	38.7 ± 22.5
%NaCl-EPS-C	1.1 ± 0.7	2.2 ± 1.5	1.6 ± 1.4
%EDTA-EPS-C	4.0 ± 1.0	4.4 ± 2.9	3.8 ± 1.9
%Bac-C	2.9 ± 3.2	7.8 ± 5.9	3.4 ± 3.7
%TEP-C	6.8 ± 6.5	6.7 ± 4.4	7.1 ± 7.3

1: mg l⁻¹; 2: µg l⁻¹; 3: mg xanthan gum eq. l⁻¹; 4: x 10⁶ ml⁻¹.

Table 2A.4 : Average concentration of the various parameters in the macroaggregate samples in different seasons.

Parameters	Monsoon	Post-monsoon	Pre-monsoon
Total macroaggregates ¹	45.7 ± 54.3	20.7 ± 12.3	40.7 ± 31.3
POC ²	1491 ± 1118	439.2 ± 328.3	1122 ± 942
Chl <i>a</i> ²	2.8 ± 1.9	3.0 ± 1.4	2.4 ± 1.8
NaCl-soluble EPS ²	29.8 ± 26.8	25.5 ± 26.8	17.3 ± 9.9
EDTA-soluble EPS ²	25.7 ± 26	22.8 ± 31.1	27.3 ± 29.6
TEP ³	13.5 ± 19.1	13.1 ± 5.2	27.2 ± 21.2
TBA ⁴	2.3 ± 2.9	3.8 ± 3.3	1.8 ± 1.5
%Phyto-C	23.6 ± 26	19.8 ± 8.2	12.1 ± 10.6
%NaCl-EPS-C	1.4 ± 1.2	2.7 ± 2.5	1.8 ± 0.9
%EDTA-EPS-C	3.2 ± 2.3	2.9 ± 2.8	1.8 ± 1.7
%Bac-C	3.8 ± 2.9	6.4 ± 6.8	3.2 ± 4.2
%TEP-C	1.4 ± 1.7	3.1 ± 2.6	1.7 ± 0.8

1: mg l⁻¹; 2: µg l⁻¹; 3: mg xanthan gum eq. l⁻¹; 4: x 10⁶ ml⁻¹.

2A.3.5 Particulate organic carbon (POC)

POC concentrations in the suspended particulate matter showed small seasonal trend. The POC concentrations ranged from 270.1 $\mu\text{g-C l}^{-1}$ (February '99) to 1311.2 $\mu\text{g-C l}^{-1}$ (April 2000) (**Table 2A.1**). During the entire sampling period, higher POC values were observed in the months of June '98 (1252 $\mu\text{g-C l}^{-1}$), November '98 (937.3 $\mu\text{g-C l}^{-1}$), August '99 (1311.2 $\mu\text{g-C l}^{-1}$) and March 2000 (1113.3 $\mu\text{g-C l}^{-1}$) (**Fig. 2A.5a**). Seasonally, POC values tend to increase with increase in suspended load (**Table 2A.3**).

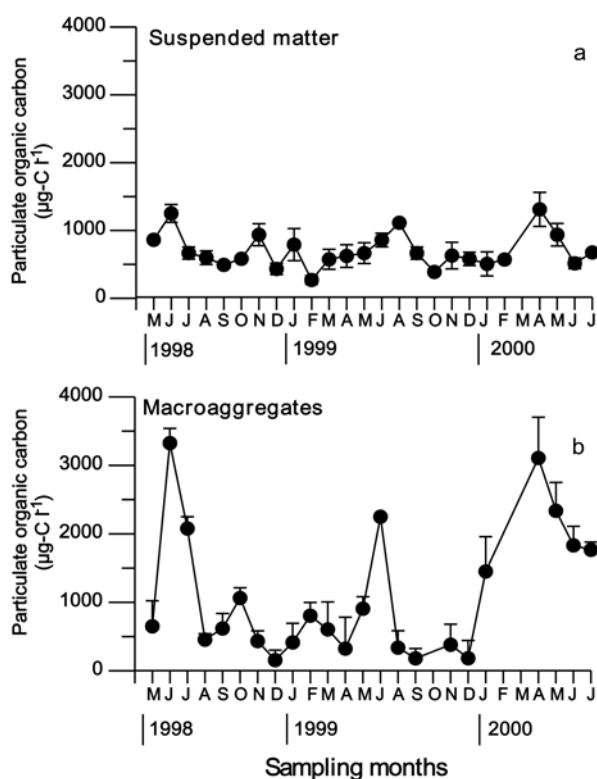


Fig. 2A.5 Seasonal variations in the concentrations of Particulate organic carbon in a) suspended matter and b) macroaggregates in the surface waters of Dona Paula Bay during 1998-2000.

On the other hand, POC concentrations in the macroaggregates showed greater variations during the sampling period. The POC concentrations in the macroaggregates ranged from 156.6 $\mu\text{g-C l}^{-1}$ (December '98) to a high of 3325.5 $\mu\text{g-C l}^{-1}$ in the month of June 1998 (**Table 2A.2**). Higher POC values were observed during June '98, October '98 (618 $\mu\text{g-C l}^{-1}$), July '99 (2250 $\mu\text{g-C l}^{-1}$) and April 2000 (3108 $\mu\text{g-C l}^{-1}$) (**Fig. 2A.5b**). The seasonal variations in the POC concentrations in the macroaggregates (**Table 2A.4**) were similar to the POC variations in the suspended particulate matter (**Table 2A.3**).

2A.3.6 Chlorophyll *a* (Chl *a*)

The Chl *a* concentrations of the suspended matter showed large scale variations during the study period (**Fig. 2A.6a**). The Chl *a* concentrations ranged from 1.2 $\mu\text{g l}^{-1}$ (July '99) to 12.3 $\mu\text{g l}^{-1}$ (November '98) (**Table 2A.1**). With the onset of monsoon (June-September), an increase in suspended load resulted in lower phytoplankton biomass ($3.8 \pm 2.5 \mu\text{g l}^{-1}$). During post-monsoon (October-January), reduced water column turbidity and higher salinity resulted in higher Chl *a* concentrations ($5.6 \pm 3.1 \mu\text{g l}^{-1}$). Similarly, higher phytoplankton biomass was also observed during pre-monsoon (February-May) months ($5.3 \pm 2.5 \mu\text{g l}^{-1}$) (**Table 2A.3**).

The seasonal variations of Chl *a* concentrations in the macroaggregate samples also showed variations during the sampling period (**Fig. 2A.6b**). Over the period of study, Chl *a* concentrations in the macroaggregates ranged from 0.4 $\mu\text{g l}^{-1}$ to 6.2 $\mu\text{g l}^{-1}$ (**Table 2A.2**). Seasonal variations of Chl *a* in the

macroaggregates were more prominent in 1998-1999 and the variations matched with the suspended Chl a concentrations.

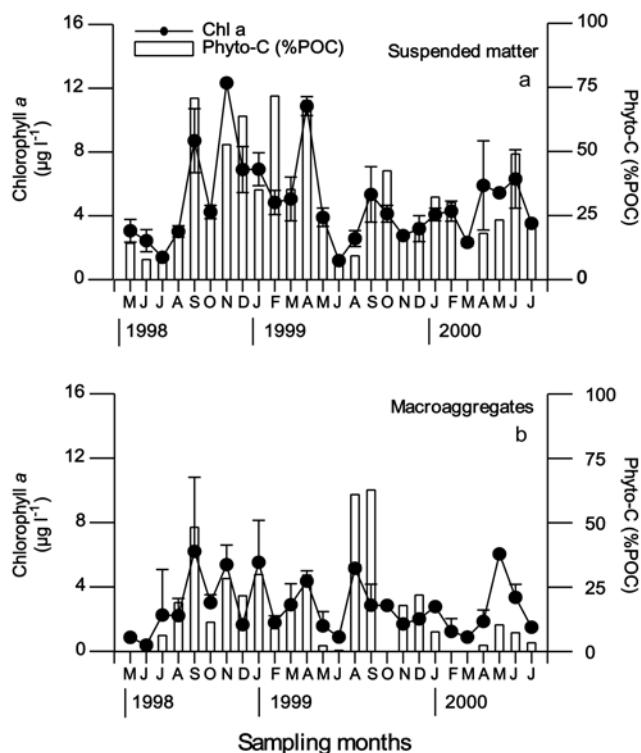


Fig. 2A.6 Seasonal variations in the concentrations of Chlorophyll a and Phyto-C (%POC) in a) suspended matter and b) macroaggregates in the surface waters of Dona Paula Bay during 1998-2000.

The phytoplankton carbon (Phyto-C) contribution was estimated using a POC/Chl a conversion factor of 40 (Banse 1977). Phyto-C was expressed as % of POC (%Phyto-C) ranged from a low of 5.5% (July '99) to 71.6% (February '99) (**Fig. 2A.6a**). The average %Phyto-C at the study site was 32.2% of the average POC (**Table 2A.1**). On the other hand, %Phyto-C in the macroaggregates ranged from a low of 0.4% (June '98) to 62.7% (September

'99) (**Fig. 2A.6b**). The average Phyto-C in the macroaggregates at the study site was 19.3% of the POC (**Table 2A.2**).

2a.3.7 Total Bacterial Abundance (TBA)

The total bacterial abundance varied from 0.5×10^6 (May '99) to 8.2×10^6 cells ml^{-1} (December '98) (**Fig. 2A.7a**). Very high abundance of bacteria was observed during post-monsoon months (October-December) in 1998-99 that declined later in the pre-monsoon months (February-May). In the following year, a similar pattern was observed but on a smaller scale.

Bacterial carbon (Bac-C) was estimated using a conversion factor of 11 fg-C cell^{-1} (Garrison et al 2000). Bac-C in the suspended matter ranged from 0.5% (April 2000) to 20.8% (December '99) (**Fig. 2A.7a**). The average contribution of Bac-C to the POC during the entire study period was 4.7% (**Table 2A.1**). The seasonal variation in bacterial carbon indicates maximum contribution during the post-monsoon (October-January) (7.8%) and minimum during the monsoon (June-September) (2.9%) (**Table 2A.3**).

The variations in bacterial abundance in macroaggregates samples were similar to that of suspended particulate matter (**Fig. 2A.7b**). The bacterial abundance varied from 0.5×10^6 cells ml^{-1} (February 2000) to 9.5×10^6 cells ml^{-1} (December '98). Seasonally, higher bacterial abundance was observed during post-monsoon (October-January) months and lower in pre-monsoon (February-May) months (**Table 2A.4**).

The Bac-C as %POC of the macroaggregates ranged from 0.1% (April '99) to 19.9% (December '99) (**Table 2A.2**). Seasonally, the Bac-C contribution to the POC was greater in 1998-99 than in the following year (**Fig. 2A.7b**). Seasonally, the Bac-C contribution was maximum (6.4%) in post-monsoon and minimum (3.2 %) in pre-monsoon (**Table 2A.4**)

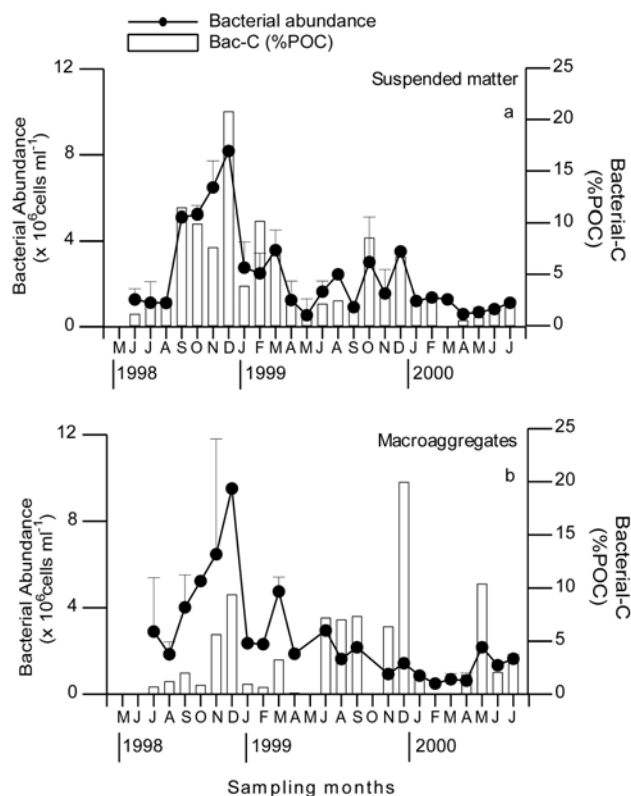


Fig. 2A.7 Seasonal variations in the abundance of total bacteria and bacterial carbon (%POC) in (a) suspended matter and (b) macroaggregates in the surface waters of Dona Paula Bay during 1998-2000.

Bacterial abundance in suspended matter had no significant relation with most of the parameters except for an inverse trend with phytoplankton biomass ($r = -0.479$) during 1999-2000 (**Table 2A.6**) and significant linear relationship with TEP concentrations during 1998-1999 ($r = 0.748$, $p < 0.001$) (**Table 2A.5**). In contrast to suspended matter, the bacterial abundance in the macroaggregate

samples had significant inverse relationship with phytoplankton biomass during May 1998 to May 1999 (**Table 2A.7**) and linear relationship with EDTA-soluble and NaCl-soluble EPS during July 1999-2000 (**Table 2A.8**).

2a.3.8 Exopolysaccharides (EPS)

NaCl-soluble EPS and EDTA-soluble EPS

There were clear differences in the distribution of NaCl-soluble EPS concentrations during the sampling period (**Fig. 2A.8a**). The NaCl-soluble EPS concentrations varied from $4.5 \mu\text{g l}^{-1}$ (March 2000) to $101.6 \mu\text{g l}^{-1}$ (January '99). The concentrations were minimal in monsoon (June-September) ($17.6 \mu\text{g l}^{-1}$) and maximal in the post-monsoon (October-January) months ($33.8 \mu\text{g l}^{-1}$) (**Table 2A.3**). In order to estimate their contribution to POC, NaCl-soluble EPS concentrations were converted to carbon by multiplying with a factor of 0.4. The NaCl-soluble EPS-C concentrations ranged from 0.4% (February 2000) to 5.4% (January '99) (**Fig. 2A.8a**) and the average carbon contribution during the study period was 1.6% of POC (**Table 2A.1**). The carbon contribution from NaCl-soluble EPS was maximal during post-monsoon months (2.2%) and minimal during monsoon months (1.1%) (**Table 2A.3**).

NaCl-soluble EPS concentrations in macroaggregates varied from $0.9 \mu\text{g l}^{-1}$ (October '98) to $84.5 \mu\text{g l}^{-1}$ (July '98) and showed an annual seasonality during the sampling period (**Fig. 2A.8b**). Seasonally, the NaCl-soluble EPS

concentrations were higher during monsoon months and lower during the pre-monsoon months (**Table 2A.4**). In the macroaggregates, the average carbon contribution from NaCl-soluble EPS was $1.7 \pm 1.7\%$ and ranged from 0.1 (July 2000) to 6.4% (January '99) (**Table 2A.2**). The variations in NaCl-soluble EPS-C of the macroaggregates showed peaks in the months of January '99 (6.4%), September '99 (3.5%) and November '99 (5.7%) (**Fig. 2A.8b**).

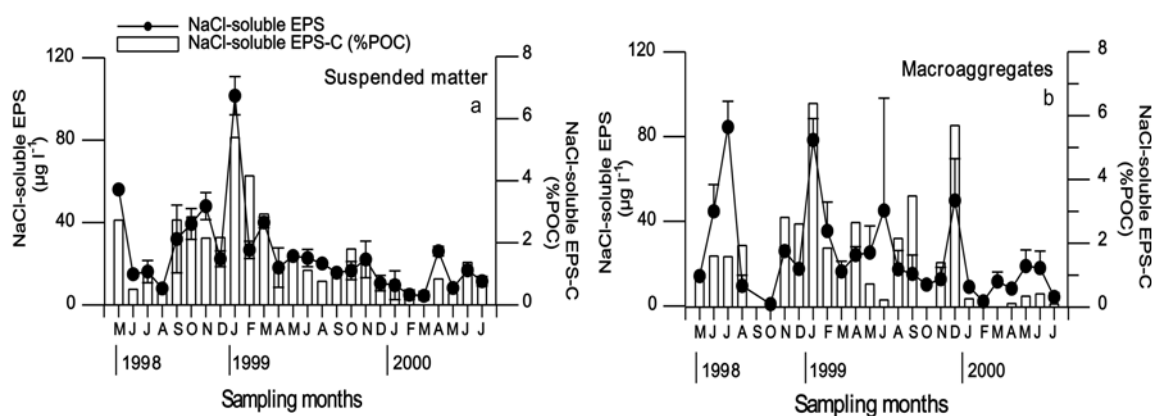


Fig. 2a.8 Seasonal variations in the concentrations of NaCl-soluble EPS and NaCl-soluble EPS-C (%POC) in (a) suspended matter and (b) macroaggregates in the surface waters of Dona Paula Bay during 1998-2000.

Concentrations of EDTA-soluble EPS during the entire study period ranged from $26.2 \mu\text{g l}^{-1}$ (February '99) to $210 \mu\text{g l}^{-1}$ (January '99) (**Table 2A.1**). Concentrations of EDTA-soluble EPS did not vary much during May 1998-99 except for the high value in the month of January '99. On the other hand, distinct variations were observed in 1999-2000, with higher concentrations

Table 2A.5. Correlation matrix of various particulate parameters of suspended particulate parameters and nutrients in the year 1998-1999.

Parameters	Chl <i>a</i>	POC	EDTA- EPS	NaCl- EPS	TEP	TBA	DO	NO ₃	PO ₄
Suspended load	-0.533	0.599*	-0.034	-0.080	-0.217	-0.517	-0.220	0.746***	0.271
Chl <i>a</i>		-0.081	-0.155	-0.192	0.169	0.242	0.220	-0.556*	0.292
POC			0.100	0.211	0.300	0.174	0.057	0.279	0.144
EDTA-EPS				0.588*	-0.163	0.106	0.079	-0.137	-0.038
NaCl-EPS					-0.123	0.285	-0.242	-0.174	-0.166
TEP						0.748***	0.301	-0.170	-0.200
TBA							0.515*	-0.475	-0.510*

*= $p < 0.05$ & ***= $p < 0.001$

Table 2A.6: Correlation matrix of various particulate parameters of suspended particulate parameters and nutrients in the year 1999-2000.

Parameters	Chl <i>a</i>	POC	EDTA- EPS	NaCl- EPS	TEP	TBA	DO	NO ₃	PO ₄
Suspended load	-0.181	0.217	0.46	0.006	-0.179	-0.236	0.606**	-0.059	0.457
Chl <i>a</i>		0.188	0.072	-0.017	0.227	-0.479	-0.189	-0.047	0.304
POC			0.482	0.620**	-0.681**	-0.198	-0.013	-0.311	0.248
EDTA-EPS				0.043	-0.352	-0.153	-0.105	-0.138	0.704***
NaCl-EPS					-0.283	0.008	-0.288	0.071	-0.271
TEP						-0.167	-0.093	0.110	-0.306
TBA							0.083	0.478	-0.288

= $p < 0.01$ & *= $p < 0.001$

Table 2A.7. Correlation matrix of various particulate parameters of macroaggregates and nutrients in the year 1998-1999.

Parameters	Chl a	POC	EDTA- EPS	NaCl- EPS	TEP	TBA	DO	NO ₃	PO ₄
Total macroaggregates	-0.281	0.496	0.379	0.332	-0.046	0.314	-0.266	0.483	0.175
Chl a		-0.472	0.226	0.008	-0.332	-0.610**	0.401	-0.453	-0.083
POC			0.248	0.654**	0.086	0.462	0.063	0.723***	0.115
EDTA-EPS				0.214	-0.165	0.067	-0.174	-0.006	-0.05
NaCl-EPS					0.021	0.107	0.458	0.609	0.122
TEP						0.342	-0.146	0.119	-0.059
TBA							-0.349	0.153	-0.002

= p<0.01 & *= p<0.001

Table 2A.8: Correlation matrix of various particulate parameters of macroaggregates and nutrients in the year 1999-2000.

Parameters	Chl a	POC	EDTA- EPS	NaCl- EPS	TEP	TBA	DO	NO ₃	PO ₄
Total macroaggregates	-0.126	0.616	0.459	0.755***	0.017	0.563	0.578*	-0.074	0.328
Chl a		-0.059	0.145	-0.169	-0.234	0.284	-0.397	-0.023	0.669**
POC			0.2166	0.0234	0.3339	0.0066	0.405	0.385	0.320
EDTA-EPS				0.4795	-0.5018	0.602*	0.314	-0.246	0.269
NaCl-EPS					-0.2029	0.687**	0.569	-0.056	-0.009
TEP						-0.470	-0.0181	-0.076	-0.331
TBA							0.222	0.362	0.328

*= p<0.05, **= p<0.01 & ***= p<0.001

during monsoon (August '99) and pre-monsoon months (May 2000) (**Fig. 2A.9a**). The %EDTA-soluble EPS-C during the study period ranged from 1.4% (April 2000) to 11.2% (January '99) (**Fig. 2A.9a**). The %EDTA-soluble EPS-C values did not vary much over the seasons (**Table 2A.3**).

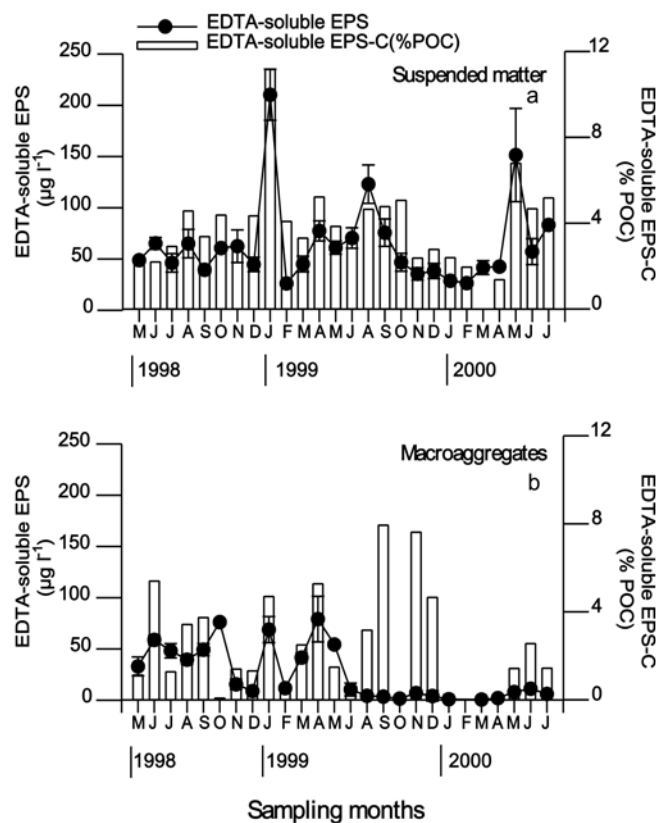


Fig. 2A.9 Seasonal variations in the concentrations of EDTA-soluble EPS and EDTA-soluble EPS-C (%POC) in (a) suspended matter and (b) macroaggregates in the surface waters of Dona Paula Bay during 1998-2000.

The EDTA-soluble EPS concentrations of macroaggregate samples ranged from $0.1 \mu\text{g l}^{-1}$ (February '99) to $79.2 \mu\text{g l}^{-1}$ (October '98) and the mean EDTA-soluble EPS concentration was $25.3 \pm 26.7 \mu\text{g l}^{-1}$ (**Table 2A.2**). Distinct differences were observed in the concentration of EDTA-soluble EPS

between 1998-1999 and 1999-2000 (**Fig. 2A.9b**). The mean EDTA-soluble EPS concentrations were similar during monsoon (June-September) ($25.7 \pm 22.7 \mu\text{g l}^{-1}$), post-monsoon (October-January) ($22.8 \pm 12.3 \mu\text{g l}^{-1}$) and pre-monsoon (February-May) season ($28.8 \pm 30.1 \mu\text{g l}^{-1}$). In the macroaggregates, the EDTA-soluble EPS-C as %POC ranged from 0.1% (January 2000) to 7.9% (September '99) during the study period (**Fig. 2A.9b**). The contribution of EDTA-soluble EPS-C was higher during monsoon months and lower during pre-monsoon months (**Table 2A.4**).

The correlation of the two types of EPS was highly variable and behaved differently in the two years. Both NaCl-soluble EPS and EDTA-soluble EPS concentrations in the suspended matter covaried with each other during 1998-1999 ($r = 0.588$; $p < 0.05$) but not in 1999-2000 (**Tables 2A.5 & 2A.6**). Moreover, there was no correlation of both forms of EPS with TEP or phytoplankton biomass. In contrast to the suspended particulate matter, NaCl-soluble EPS concentrations in the macroaggregates had no significant correlation with EDTA-soluble EPS during the sampling period (**Table 2A.7 & 2A.8**). Similarly, both forms of EPS did not show any significant correlation with phytoplankton biomass (**Table 2.7 & 2.8**). However, the two forms showed positive linear trends with total macroaggregate concentration and inverse trends with TEP concentrations.

TEP concentrations (Alcian blue method)

TEP concentrations in the suspended matter samples ranged from 1.3 (July '99) to 149.1 μg Xanthan gum eq. l^{-1} (June 2000) during the entire study period (**Fig. 2A.10a**) and the average concentration was 55.6 ± 35.6 μg Xanthan gum eq. l^{-1} (**Table 2A.1**). There were no seasonal differences in the average TEP concentration for monsoon, post-monsoon and pre-monsoon

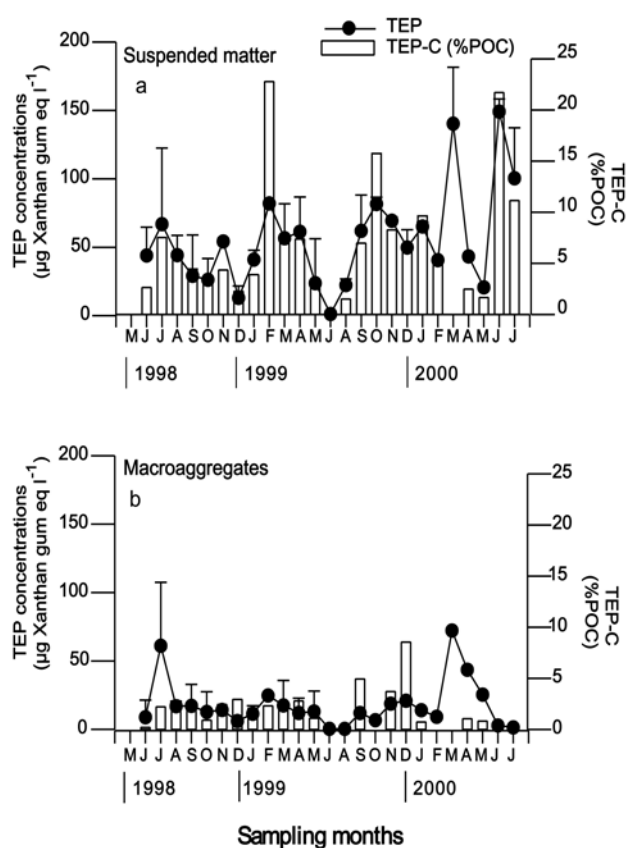


Fig. 2A.10 Seasonal variations in the concentrations of TEP and TEP-C (%POC) in a) suspended matter and b) macroaggregates in the surface waters of Dona Paula Bay during 1998-2000.

months (**Table 2A.3**). The TEP-C values expressed as % of POC (%TEP-carbon) ranged from 0.1% (July '99) to 22.8% (February '99) (**Fig. 2A.10a**).

The average TEP-carbon contribution in the suspended matter was 6.9% (**Table 2A.1**) and showed no major seasonal differences (**Table 2A.3**).

TEP concentrations in the macroaggregate samples ranged from 0.4 (August '99) to 72.3 μg Xanthan gum eq. l^{-1} (March '99) (**Table 2A.2**). TEP concentration showed small-scale variations throughout the study period and remained <25 μg Xanthan gum eq. l^{-1} except for the high values observed in July '98 and March '99, respectively (**Fig. 2A.10b**). Seasonally, higher TEP concentrations were observed during pre-monsoon months than in the other two seasons (**Table 2A.4**). The %TEP-C in macroaggregates ranged from 0.01 % (July '99) to 8.5% (December '99) (**Table 2A.2**). The seasonal TEP carbon contribution was greater in the post-monsoon season compared to the other two seasons (**Table 2A.4**).

TEP concentrations in the suspended matter did not have any correlation with phytoplankton biomass and either form of EPS during the sampling period (**Table 2A.3 & 2A.4**). However, TEP had significant linear relation with bacterial abundance ($r=0.748$; $p<0.001$) during 1998-1999 only. On the other hand, TEP concentrations in the macroaggregate samples had no correlation with the other particulate parameters during 1998-99 (**Table 2A.5**). In the following year (1999-2000), TEP had weak inverse trend with EDTA-soluble EPS concentrations (**Table 2A.6**) but no definite trend between TEP concentrations and bacterial abundance.

2A.4 Discussion

EPS and TEP dynamics

Both forms of EPS (NaCl-soluble EPS and EDTA-soluble EPS) in suspended matter and macroaggregates showed temporal and seasonal variations. The concentrations of NaCl-soluble EPS and EDTA-soluble EPS in the suspended matter varied from 4.5 to 101.6 $\mu\text{g l}^{-1}$ and 26.2 to 210.3 $\mu\text{g l}^{-1}$, respectively. Similarly, NaCl-soluble EPS and EDTA-soluble EPS concentrations in the macroaggregates varied from 0.9 to 84.6 $\mu\text{g l}^{-1}$ and 0.01 to 76.2 $\mu\text{g l}^{-1}$, respectively. Various factors like phytoplankton biomass, phytoplankton bloom composition, age of the bloom, microbial activity, nutrients and inputs from benthic phytoplankton can influence the concentrations and seasonality of EPS in natural environments (Chrost and Faust 1983, Herndl 1993, Obernosterer and Herndl 1995, Kepkay et al 1997).

Seasonal variations of NaCl-soluble EPS and EDTA-soluble EPS in suspended matter and macroaggregates did not covary with those observed for phytoplankton biomass. The study site experienced higher phytoplankton biomass during the months of September-October, December to February and April (**Fig. 2A.6**). Microscopic observation of the samples revealed that species of *Coscinodiscus*, *Skeletonema*, *Nitzschia* and *Leptocylindrus* constituted bulk of the phytoplankton population during September-October, whereas *Chaetoceros* and *Nitzschia* were the dominant genera during months of April and June. However, both NaCl-soluble EPS and EDTA-soluble EPS values peaked only in the month of January '99. A similar trend could be

observed only for the NaCl-soluble EPS in the macroaggregates during 1998-99. Our data suggests that the variation in EPS concentrations in suspended matter was not reflected in the macroaggregates. Similarly, higher values of EPS did not match with the higher Chl *a* values in the suspended matter and macroaggregates. This was also evident from the relatively poor relationship between EPS and Chl *a* concentrations (**Tables 2A.5, 2A.6, 2A.7 & 2A.8**). Kepkay et al (1997) observed that production of extracellular compounds like EPS may not always match with Chl *a* concentrations or phytoplankton cell abundance, especially in mixed blooms as observed at the study site. Moreover, the production and nature of EPS produced shall vary with species and the dominant species need not always be the major contributor to EPS in the water column. The variable correlation of EPS with other particulate parameters like suspended load and POC in the water column (**Tables 2A.5 and 2A.6**) and total macroaggregates in the macroaggregate samples also indicates that the dynamics of EPS at the study site was not entirely controlled by phytoplankton biomass alone.

Exceptionally high values of NaCl-soluble EPS in both suspended matter and macroaggregates and EDTA-soluble EPS in the suspended matter were observed in January '99. Heavy growth of seaweeds like *Sargassum* was observed at the study site between December to February. It has been reported that brown algae like *Sargassum* release high molecular weight mucus exudates during their growth (Tanaka and Asakawa 1988). It is possible that the mucus released by the seaweeds growing in the study site

might have contributed to the exceptionally high EPS concentrations during January '99.

Nutrient concentrations may also play an important role in regulation of EPS concentrations in the water column. During their growth, phytoplankton produces more extracellular organic matter under nutrient-especially nitrate, limiting conditions (Myklestad 1995). Weak inverse trends could be established between nutrients and EPS concentrations in the suspended particulate matter (**Table 2A.3 & 2A.4**). In contrast, dissolved nitrate did not show any inverse trends with NaCl-soluble EPS during the sampling period. On the other hand, EDTA-soluble EPS showed weak negative correlation with nitrate concentrations during 1999-2000 (**Table 2A.5 & 2A.6**). Thus it was evident that the influence of variations in nutrient concentrations on the distribution of EPS was not evident at the study site.

EPS in shallow water stations can come from phytobenthos of the sediments. Since resuspension of bottom sediments is a regular phenomenon in such waters, a close association between EPS and suspended load would be expected. In the present study, suspended load did not have any significant correlation with either NaCl-soluble EPS or EDTA-soluble EPS (**Table 2A.3 & 2A.4**). Thus, neither phytoplankton nor resuspended sediment was a dominant source of EPS in the suspended particulate matter at the study site. On the other hand, EPS in macroaggregates had linear trends with the total macroaggregates (**Table 2A.5 & 2A.6**). From the total macroaggregate data (**Fig 2A.4b**), it was clear that total macroaggregate concentrations were

higher during the monsoon months (June to September). Thus it is possible that resuspended particulate matter might contribute to the total macroaggregate concentrations. Moreover, the poor correlation between EPS and Chl *a* concentrations suggest that EPS in the macroaggregates was not of phytoplankton origin. Since the station is very shallow and close to the shore, its possible that EPS in the macroaggregates might have alternate sources including benthic inputs.

Another important factor that might regulate EPS dynamics is microbial activity. Both laboratory (Smith et al 1995, Goto et al 2001) and field studies (Reibesell 1991, Janse et al 1999) have shown that bacterial activity can hydrolyze EPS to meet their carbon demand. The total bacterial abundance in the suspended matter did not show any correlation with either NaCl-soluble EPS or EDTA-soluble EPS concentrations (**Table 2A.3 & 2A.4**). In contrast, EPS had a better correlation with bacterial abundance in macroaggregates during 1999-2000 (**Table 2A.6**). Macroaggregates are enriched in organic matter like EPS, phytoplankton cells etc. (Herndl 1993). EPS adsorbed to macroaggregates might be actively degraded by particle-associated bacteria (Karner and Herndl 1992, Agis et al 1998, Grossart and Simon 1998, Unanue et al 1998, Worm and Sondergaard 1998). Moreover, a tight coupling between the organic matter and the bacterial abundance has been reported in macroaggregates (Goldman 1984). The significant linear correlations in the macroaggregates (**Table 2A.6**) suggest that accumulation of EPS appeared to support bacterial growth. On the other hand, the difference in correlations of EPS and bacterial abundance between 1998-1999 and 1999-2000 might be

due to differences in composition of bacterial community and quality of the EPS (Herndl 1993, Decho and Herndl 1995).

In the natural environments, increase in the production of EPS and TEP coincide with termination of phytoplankton blooms (Passow et al 1994). However, a similar phenomenon was not evident at the study site. In the present study, TEP concentrations in the water column were generally higher during the monsoon and pre-monsoon months during which the phytoplankton biomass was relatively low (**Table 2A.1**). The concentration of TEP in shallow water environments might be regulated by various factors including phytoplankton biomass and bacterial activity (Alldredge and Passow 1993, Mari and Kjørboe 1996), land run-off, terrestrial inputs and resuspended sediments (Jahmlich et al 1998, Passow 2002). The higher TEP concentrations during monsoon months might have been due to an increase in resuspended sediments. However, inverse trends between TEP and suspended load indicate that suspended load did not significantly influence the TEP concentrations. On the other hand, fresh water runoff brings in organic matter in the study area, which leads to increase in both dissolved and particulate organic carbon (Qasim and SenGupta 1981). Moreover, TEP concentrations had only a weak positive trend with Chl *a* concentrations (**Table 2A.5 & 2A.6**) and weak negative correlation with EPS (both NaCl-soluble and EDTA-soluble). A similar correlation is also observed in the macroaggregate data (**Table 2A.7 & 2A.8**). Microbial EPS, dissolved organic carbon (DOC) and cell lysate may act as a source of TEP in the aquatic environments (Herndl 1995, Alldredge and Jackson 1995, Passow 2002).

However in shallow coastal waters like Dona Paula Bay, additional inputs from allochthonous sources might also play an equally important role in TEP distribution as observed from the variable correlation of TEP concentrations with EPS and Chl *a* in both the suspended matter and macroaggregates.

TEP are rich in polysaccharides and hence may act as a carbon source for bacteria. In the surface water samples, bacterial abundance had linear correlation with TEP only in the year 1998-99 (**Table 2A.3**). In the following year, the relation was insignificant and inverse. Such a mixed relation could not be explained using the available data. On the other hand, a weak positive correlation was also observed between TEP concentrations and bacterial abundance in the macroaggregates (**Table 2A.5 & 2A.6**). Such correlations in the suspended matter and macroaggregate samples suggest that increase in TEP might be the result of bacterial activity in the water column. If TEP were to serve as substrate for bacterial growth, the TEP concentration should have decreased with increasing bacterial abundance. However, such a relationship was not evident at the study site. Thus, it appears that TEP at the sampling site might be inert to microbial degradation. However, the inert nature of TEP could not be substantiated in the absence of data on heterotrophic activity in the Dona Paula bay.

Contribution of EPS-C to the POC

In the productive coastal waters, phytoplankton followed by bacteria, EPS and TEP together form a major source of POC. Earlier studies in the Dona Paula

Bay have shown that extracellular organic carbon released during phytoplankton blooms contributed about 4 – 38% of the primary production (Gomes et al, 1991). The estimates of EDTA-soluble EPS -C, NaCl-soluble EPS -C and TEP-C contribution to the total POC in the surface waters indicate that TEP-C (6.9%) was second major contributor to the POC after phytoplankton-C. On the other hand, NaCl-soluble EPS-C and EDTA-soluble EPS-C contribution to the POC was 1.7% and 4%, respectively. Assuming 80% removal of EPS by bacteria (Gomes et al 1991), the sum of actual EPS-C contribution would be ~9% of POC. Thus, TEP-C contribution was relatively higher than the sum of the contribution from EDTA-soluble EPS-C (4%) and NaCl-soluble EPS-C (1.7%) and Bact-C (4.7%). On the other hand, the average TEP-C contribution was 21% of the average Phyto-C (32.2% of POC). In comparison to suspended matter, TEP-C in macroaggregates was much less but still greater than %NaCl-soluble EPS-C of the aggregates. However, there was little difference between TEP-C and total carbon contribution of EPS. It is interesting to note that TEP-C did not appear to support bacterial biomass. Although %TEP-C was not very high, this fraction might act as a sink of organic carbon in these waters. However, further studies need to be carried out to ascertain the fate of TEP-C in these waters.

2A.5 Conclusions

A distinct seasonal variation in the concentrations of EPS and TEP was observed in both suspended particulate matter and macroaggregate samples collected from Dona Paula Bay. The concentrations of EPS and TEP and

abundance of TEP in both suspended matter and macroaggregates were controlled by both physical factors (water column turbidity) and biological factors (phytoplankton biomass and bacterial abundance). EPS (except TEP) was not a major source of POC for bacterial population in the study area. The TEP-C was relatively higher than both EPS-C and bacterial carbon in the surface waters of the Dona Paula Bay. However, no relation could be established between bacterial abundance and TEP in the water column. This implies that TEP might not be a preferred carbon source for bacteria in the Dona Paula Bay.

Chapter 2B

2B. 1 Introduction

Estuaries are highly dynamic and complex ecosystems that are in a constant state of flux. The organic carbon demand in these ecosystems is met by combination of autochthonous and allochthonous sources (Peduzzi and Herndl 1992, Mann and Wetzel 1998, Conan et al 1999) and microbial activity (Fuhrman and Azam 1982, Strom et al 1997). Bacterioplankton growing in such a dynamic environment have to cope with wide fluctuations in physical (tides, salinity, temperature, turbidity etc.) (Shiah and Ducklow 1994, Jorgensen et al 1998, Wikner et al 1999, Cunha et al 2000, Almeida et al 2001) and chemical characteristics (substrate availability, substrate complexity) (Thingstad and Billen 1994, Amon and Benner 1996, Foreman et al 1998, Covert and Moran 2001) in the water column. Moreover, such variations influence the quality and quantity of the organic matter in the water column (Raymond and Bauer 2000, Søndergaard et al 2000). Such fluctuations may bring about a shift in bacterial production, activity and community structure (Janse 2000, Simon et al 2002).

One of the notable features of bacterial utilization of organic matter (both particulate and dissolved) is the production of an array of ecto-enzymes including glucosidases, peptidases and proteases, which breakdown the complex organic molecules to smaller molecules. These smaller molecules are then utilized

microorganisms for their growth (Chrost, 1989). Due to their substrate specificity, enzymes have also been used to assess the quality of the organic matter in the aquatic environments. For example, glucosidases are carbohydrate specific enzymes that cleave α - or β - 1-6 glycosidic linkages (Somville 1988) and are potentially useful to assess the quality of organic matter in the aquatic environments (Herndl 1993, Decho and Herndl 1995).

Enzyme activities have been measured either by estimating the rate of accumulation of product (Duddridge and Wainwright 1982) and the use of chromophores (Meyer-Reil 1981) or fluorogenic substrate analogs (Hoppe 1983). Fluorogenic substrate analogs are preferred for its sensitivity, enzyme specificity and accuracy and short period of incubation. These substrates have been used widely to assess the heterotrophic activity in freshwater (Middelboe and Søndergaard 1995), limnic (Munster 1991, Grossart and Simon 1998), brackishwater (Hoppe 1983), estuarine and coastal water (Ammerman 1991, Somville 1993, Almeida et al 2001, Cunha et al 2001) and pelagic environments (Cho and Azam 1988, Muller-Niklas et al 1994).

Numerous studies have been carried out to assess the enzyme activity, substrate turnover and bacterial production in oceanic (Muller-Niklas et al 1994, Christian and Karl 1995, Nausch et al 1998), estuarine (Almeida et al 2001, Cunha et al 2001), limnic (Chrost 1991, Vrba 1992, Middelboe and Søndergaard 1993) and riverine (Berger et al 1995) ecosystems of temperate waters. Although tropical

coastal waters are one of the most productive ecosystems, not much is known about bacterial abundance, production and enzyme activity in these ecosystems (Rath et al 1993).

Dona Paula Bay experiences wide fluctuations in the hydrography, nutrients and quality of organic matter (Bhaskar et al 2000). In Chapter 2a, it was shown that both autochthonous inputs like phytoplankton biomass and EPS associated with particulate matter had moderate to significant correlation with bacterial abundance, especially in the macroaggregate samples. The study area is also influenced by detrital inputs from mangroves (DOM and POM) that might support microbial carbon demand (Wafar et al 1997). Keeping in mind the above facts, a 13-month field sampling was carried out during 1999-2000 at a shallow water station in the Dona Paula Bay. The objectives were to study 1) the seasonal variation of bacterial production and ecto-enzyme activity and 2) to assess the role of organic substrates (phytoplankton biomass and EPS) on the heterotrophic activity of bacteria in seawater samples and macroaggregates.

2B. 2 Materials and Methods

Sample collection and analyses

During the year 1999-2000, seawater samples were collected as described in Chapter 2a for the analyses of hydrography (temperature, salinity) and nutrients (nitrate and phosphates). Macroaggregate samples were also collected following the method described in Chapter 2a. Both seawater and macroaggregate

samples were then filtered onto preashed GF/F filters for the analyses of various particulate parameters like dry weight, Chl *a*, POC, EPS and TEP. Sub-samples of seawater and macroaggregates were fixed with 0.22 μm filtered formalin (4% final concentration) for bacterial enumeration. Suitable sub-samples were also collected to estimate bacterial production and enzyme activity. Methods for estimation of temperature, salinity, nutrients and the particulate parameters were same as described in Chapter 2a except for bacterial production and enzyme activity, the details of which are described below.

2B.2.1 Bacterial abundance

The formalin fixed seawater and macroaggregate samples were filtered onto 0.22 μm prestained Nuclepore membrane filters and stained with 0.22 μm pre-filtered acridine orange for the enumeration of total bacterial abundance following Parsons et al (1984) as given in Chapter 2a. Additional samples were filtered through GF/F filters and the filtrate was collected in sterile glass tubes. One ml of the filtrate containing particle free bacteria, was then stained with acridine orange, filtered onto 0.22 μm prestained Nuclepore membrane filters and enumerated as described in Chapter 2a. Particle associated bacteria (PAB) was calculated as the difference between total and particle free bacterial population.

2B.2.2 Bacterial production

Working solution of thymidine

A working solution of $^3\text{H}_1$ -thymidine was prepared by drying one ml of $^3\text{H}_1$ -thymidine stock solution (1mCi; Sp. activity: 12000 mCi mmole⁻¹) under a stream of nitrogen. The dried thymidine was then diluted in 3.3 ml deionized water to obtain a working solution of 5 nmole ml⁻¹ thymidine concentration.

Methodology

The bacterial production rates in the seawater and macroaggregate samples were estimated from the incorporation rates of $^3\text{H}_1$ -thymidine. The samples were analyzed following the JGOFS protocol (1994). In order to estimate total bacterial production, ten ml of unfiltered seawater and macroaggregates samples in triplicate were dispensed in test-tubes. Twenty μl working solution of thymidine was added (10 nM thymidine final concentration) to the test-tubes. Similarly, one set of sample was fixed with filtered formalin (4% final concentration) prior to addition of $^3\text{H}_1$ -thymidine and treated as control. Both sets were incubated in dark for 60 mins at room temperature. In order to estimate the particle free bacterial production, 10 ml of both seawater and macroaggregate samples filtered through preashed GF/F filters at <10 mbar pressure, was inoculated with 20 μl of $^3\text{H}_1$ -

thymidine and estimated for particle free bacterial production as mentioned above.

At the end of incubation period, uptake of $^3\text{H}_1$ -thymidine was terminated with the addition of 0.22 μm filtered formalin (4% final concentration). The samples were filtered at <10 mbar pressure through 0.22 μm cellulose nitrate Millipore filters pretreated with ice-cold 5% trichloroacetic acid (TCA). The filter papers were then rinsed thrice with 5% ice-cold TCA, followed by three rinses of ice-cold ethanol and were filtered to dryness. The filter paper was then transferred to clean scintillation vials and completely dissolved in 2 ml ethyl acetate. Four ml of scintillation cocktail was added to each sample and allowed to stabilize overnight. The replicate samples were counted over one minute using a scintillation counter (Wallac 2000).

The thymidine uptake rate was calculated using the following equation:

$$[\text{methyl-}^3\text{H-thymidine}] \text{ nM h}^{-1} = \frac{(\text{dpm}_{\text{sample}} - \text{dpm}_{\text{blank}})}{2200} \times \frac{1000}{V} \times \frac{1}{\text{SA}} \times \frac{60}{T}$$

where dpm is disintegrations per minute; 2200 is dpm per nCi; V is extraction volume, SA is the specific activity and T is incubation time (hours).

The thymidine uptake rate was converted to bacterial production (Bp) using a conversion factor (CF) of 2×10^{18} cells per mmole of thymidine utilised (Riemann et al. 1987) as given below:

Bacterial production (Bp) ($\mu\text{g-C l}^{-1} \text{ h}^{-1}$) = Thymidine counts x CF x carbon cell⁻¹;

where carbon cell⁻¹ was assumed to be 11 femto g (Garrison et al 1999). Cell-specific production (SBp) was calculated as below:

$$\text{SBp (femto g-C cell}^{-1} \text{ h}^{-1}) = \frac{\text{Bacterial production}}{\text{Bacterial abundance}}$$

2B.2.3 Ecto-glucosidase activity

Working solution of MUF-substrates

The working solution was prepared using 1:1 dilution of 100 μM substrate analogs (4-methyl umbelliferryl (MUF) α - and β -glucoside) stock solution (33.8 $\mu\text{g ml}^{-1}$) in deionized water. The substrate standard stock was kept frozen at -20°C and fresh working solution was prepared before each sample analyses.

The ecto-glucosidase activity in the seawater and macroaggregate samples was estimated using (MUF) α - and β -glucosides (Hoppe 1983 & 1993). The fluorescent substrate analogs are substrates that are linked to artificial fluorochromes by specific bindings. When added to a natural sample, these substrates are enzymatically cleaved and the amount of fluorochromes released is used as a direct measure of the microbial enzyme activity.

Calibration

The method was calibrated for the required substrate concentration and incubation period for both MUF- α and β -glucoside. Briefly, substrate solution of increasing concentrations (5, 10 and 20 nM final concentration) were added to unfiltered seawater samples in triplicate and incubated at room temperature ($28 \pm 2^\circ\text{C}$) up to 10 h. Sub-samples were removed after every 2 h and the fluorescence of the substrate was measured. Calibration of fluorochrome was carried out using increasing concentrations of MUF (methyl umbelliferone) standard (2, 4, 6, 8, 10 & 20nM). A factor was calculated by plotting the fluorescence intensity against the concentration and was later used in the calculation of the enzyme activity.

Experimental setup

In order to estimate the total ecto-glucosidase activity (both α - and β -glc), 5 μl (50 μM) MUF- α & β glucoside was added to 25 ml of surface seawater and macroaggregate samples (10 nM final concentration) dispensed into 50 ml Ehrlenmeyer flasks. Similarly, 5 μl of 50 μM MUF- α & β glucoside was added to 25 ml of GF/F filtered surface seawater and macroaggregates dispensed in 50 ml Ehrlenmeyer flasks to estimate the particle free ecto-glucosidase activity. The particle associated ecto-glucosidase activity was calculated as the difference between total and particle free glucosidase activity. In order to estimate the dissolved cell-free glucosidase activity, MUF-substrates were added to seawater

samples filtered through 0.22 μm cellulose nitrate filters. One set of unfiltered samples was heat killed (100°C) prior to addition of MUF-substrate and used as blank. Both the samples and blanks were incubated in dark at room temperature ($28 \pm 2^{\circ}\text{C}$) for a period of 2 to 5 h.

After incubation, 1ml of the sample was transferred into a cuvette containing 100 μl of Tris-HCl buffer (pH 10.3), mixed properly and measured at 365 nm excitation and 460 nm emission wavelengths using a fluorescence spectrophotometer (Hitachi F-2000).

The rate of enzyme activity was calculated as follows:

$$\text{nM C h}^{-1} = (f_t - f_0) \times F \times \text{carbon content of the substrate};$$

where $f_t - f_0$ is the difference in fluorescence between final time t_f and incubation time t_0 ; F is the standard factor (Standard concentration/Standard fluorescence) obtained from calibration curve and carbon content was $72 \mu\text{g C } \mu\text{M}^{-1}$ (Hoppe, 1993). The cell-specific activity (femto mol-C cell $^{-1}$ h $^{-1}$) was calculated as below:

$$\text{Cell-specific activity (Sp- activity)} = \frac{\text{Total enzyme activity}}{\text{Total bacterial Abundance}}$$

2B.2.4 Statistical Analyses

Pearson correlation coefficients were obtained between various particulate parameters and bacterial abundance, bacterial production and enzyme activity in both seawater samples and macroaggregate samples. In order to assess the

influence of the particulate matter composition on the microbial activity in the macroaggregates, correlations were obtained between the bacterial production and enzyme activity of macroaggregate samples with various particulate parameters of the seawater samples. The closeness of the particulate parameters on the microbial activity was further evaluated using Principal component analysis method after extracting the factor loadings.

2B.3 Results

2B.3.1 Calibration

The calibration for optimal incubation time and optimal substrate concentrations required to estimate the ecto-glucosidase activity is shown in **Fig. 2B.1a** and **Fig. 2B.1b**, respectively. It was found that substrate concentration of 10 nM and an incubation time of 2 h was optimal. These optimal conditions were employed to evaluate the enzyme activity at the Dona Paula Bay.

2B.3.2 Bacterial Abundance

Total bacterial abundance (TBA) in the seawater samples varied from 0.6×10^6 cells ml⁻¹ (April 2000) to 3.5×10^6 cells ml⁻¹ (December '99) during the sampling period (**Fig. 2B.2a**). TBA showed an increasing trend from July to December '99 followed by a sharp decline for the remaining period of sampling.

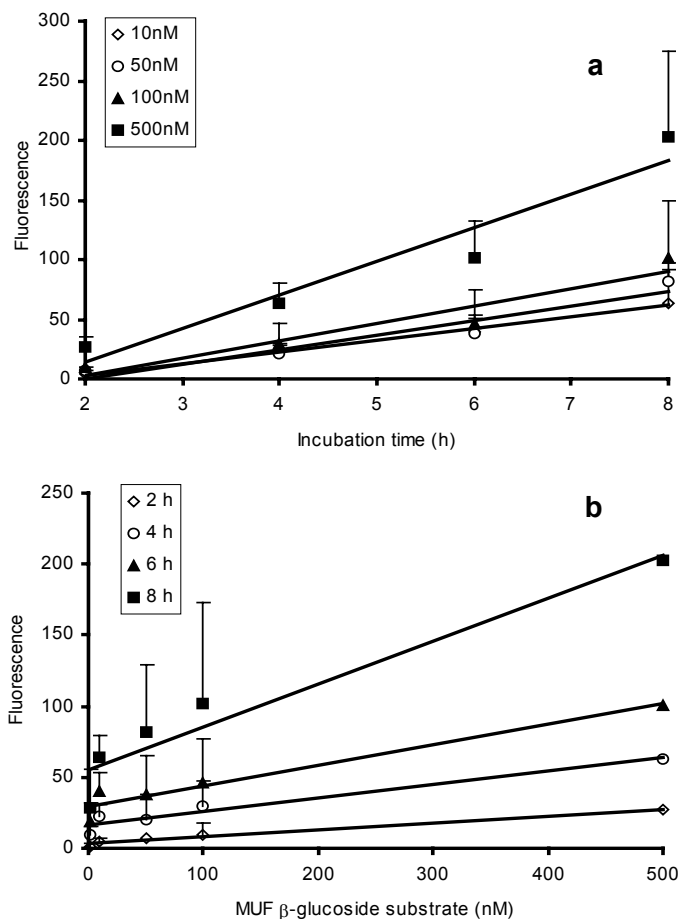


Fig. 2B.1 Linear plots showing the effect of incubation time (a) and MUF-substrate concentrations (b) on b-glucosidase activity. A substrate concentration of 10 nM ($r^2 = 0.994$) with an incubation period of 2 hours ($r^2 = 0.978$) was optimum to estimate the enzyme activity.

The total bacterial abundance in macroaggregates varied from 0.5×10^6 cells ml^{-1} (February 2000) to 2.9×10^6 cells ml^{-1} (July '99) during the sampling period (**Fig. 2B.2b**). The bacterial abundance declined from July '99 until February 2000 followed by a small increase for the remaining period of observation.

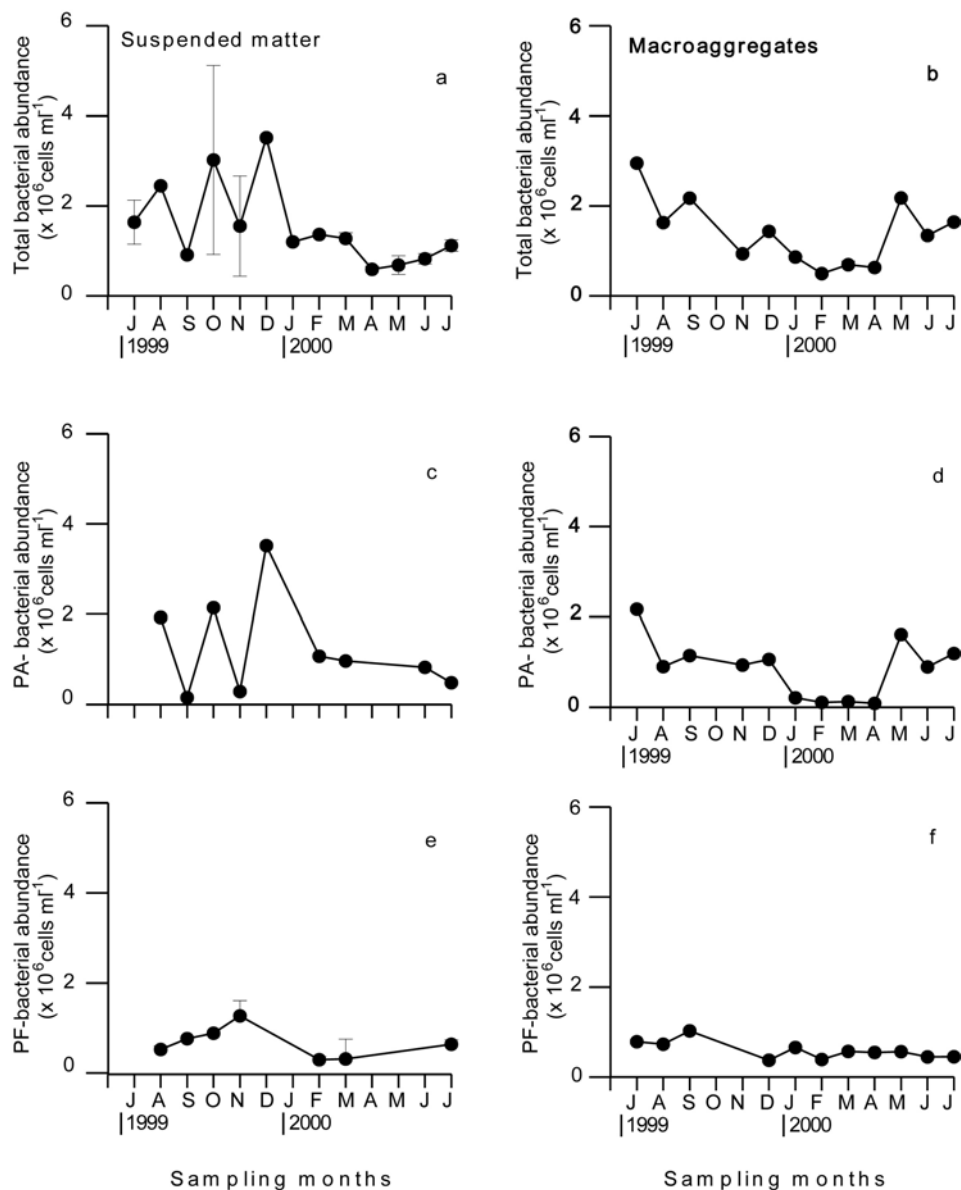


Fig. 2B.2 Temporal variations of Total bacterial abundance (a & b), Particle associated bacterial abundance (c & d) and Particle Free-living bacterial abundance (e & f) in the the suspended matter and macroaggregate samples collected from Dona Paula bay during 1999-2000. Each point is the average of duplicate counts and the error bars indicate the SD.

Particle associated bacterial abundance (PAB) in the seawater samples ranged from 0.1×10^6 cells ml⁻¹ (September '99) to 3.4×10^6 cells ml⁻¹ (December '99)

(**Fig. 2B.2c**). On the other hand, PAB in macroaggregates ranged from 0.1×10^6 cells ml^{-1} (April 2000) to 2.2×10^6 cells ml^{-1} (July '99), respectively (**Fig. 2B.2d**). On an average, particle associated bacteria in seawater and macroaggregate sample was $1.3 \pm 1.1 \times 10^6$ cells ml^{-1} and $0.9 \pm 0.7 \times 10^6$ cells ml^{-1} (**Table 2B.1 and 2B.2**), which accounted for 54% and 64% of the total bacterial abundance, respectively. Particle free bacterial (PFB) abundance in seawater and macroaggregate samples varied from 0.3 to 1.3×10^6 cells ml^{-1} (**Fig. 2B.2e**) and 0.4 to 1.0×10^6 cells ml^{-1} (**Fig. 2B.2f**) during the sampling period. Moreover, the counts were relatively higher in 1999 than in 2000. However, no specific trend was observed in the seasonality of the PFB in both suspended matter and macroaggregate samples.

2B.3.3 Bacterial production

The total bacterial production (TBp) ranged from $0.4 \mu\text{g-C l}^{-1}\text{h}^{-1}$ (November '99) to $3.2 \mu\text{g-C l}^{-1}\text{h}^{-1}$ (June 2000) (**Fig. 2B.3a**) and showed large-scale variations without any definite trend during the sampling period. TBp increased from $0.5 \mu\text{g-C l}^{-1}\text{h}^{-1}$ (August '99) to $3.1 \mu\text{g-C l}^{-1}\text{h}^{-1}$ (September '99), followed by a similar higher bacterial production during the months of December '99 ($2 \mu\text{g-C l}^{-1}\text{h}^{-1}$) and June 2000 ($3.2 \mu\text{g-C l}^{-1}\text{h}^{-1}$) (**Fig. 2B.3a**). The specific bacterial productivity (SBp) ranged from 0.3 femto $\text{g-C cell}^{-1}\text{h}^{-1}$ (May 2000) to 2.6 femto $\text{g-C cell}^{-1}\text{h}^{-1}$ (April 2000) and the average specific production was 1.2 ± 0.8 femto $\text{g-C cell}^{-1}\text{h}^{-1}$ during the entire sampling period (**Table 2B.1**).

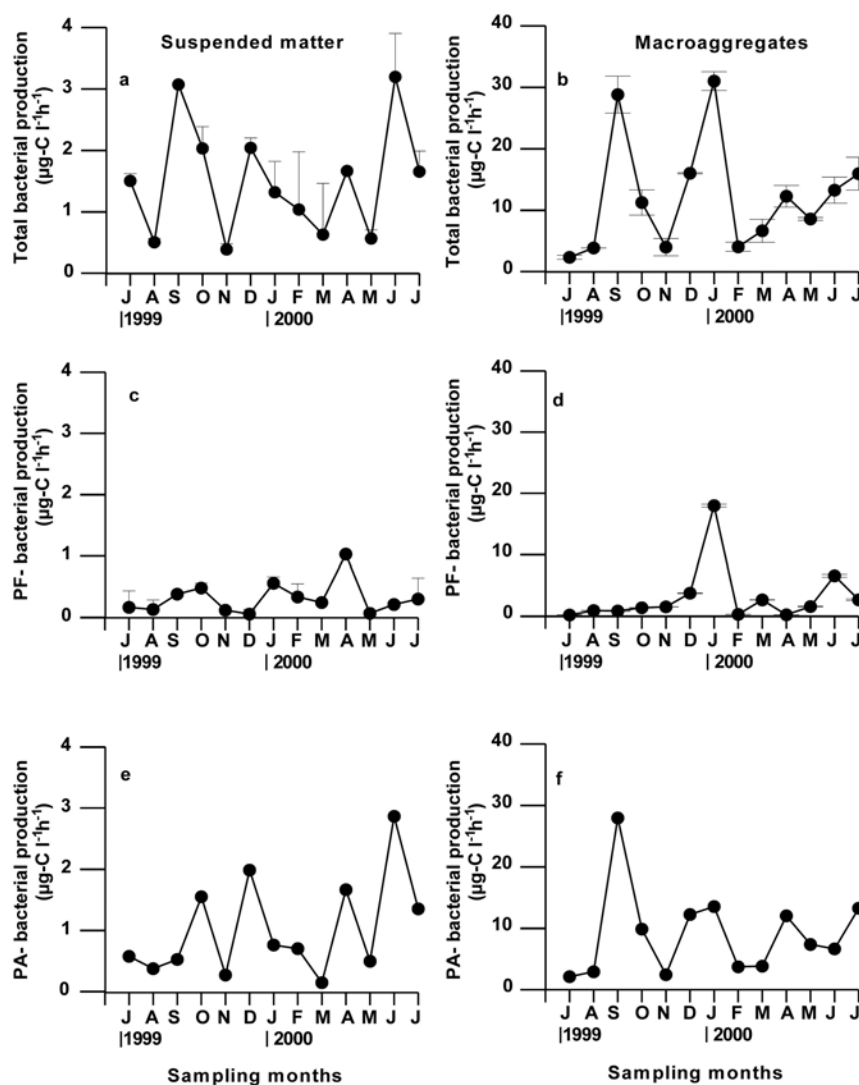


Figure 2B.3. Temporal variations of Total bacterial production, Particle Free bacterial production and Particle Associated Bacterial production in the suspended matter (a, c & e) and macroaggregates (b, d & f) in the surface waters of Dona Paula Bay during 1999-2000. Each point is mean of duplicate samples and the error-bars indicate SD.

Table 2B.1: Mean, Sd and range of bacterial abundance, production, ecto-glucosidase activity, specific bacterial production, specific enzyme activity and enzyme ratio in the seawater samples during 1999-2000.

Parameters		Mean	SD	Max	Minimum
TBA	$\times 10^6$ cells ml^{-1}	2.4	2.0	3.5	0.6
PAB	$\times 10^6$ cells ml^{-1}	1.3	1.1	3.4	0.1
TBp	$\mu\text{g-C l}^{-1} \text{h}^{-1}$	1.6	1.0	3.2	0.4
PABp	$\mu\text{g-C l}^{-1} \text{h}^{-1}$	1.2	1.0	3.1	0.2
SBp	femto g-C $\text{cell}^{-1} \text{h}^{-1}$	1.2	0.8	2.6	0.3
Total α -glc	nM-C h^{-1}	22.6	32.6	127.5	2.3
PA- α -glc	nM-C h^{-1}	19.2	26.2	101.4	1.6
PF- α -glc	nM-C h^{-1}	3.7	7.2	26.1	0.1
Total β -glc	nM-C h^{-1}	17.0	15.2	62.4	3.0
PA- β -glc	nM-C h^{-1}	13.8	10.4	39.8	3.0
PF- β -glc	nM-C h^{-1}	4.2	6.7	22.6	0.1
α/β ratio		1.1	0.4	2.0	0.5
Sp- α -glc activity	femto mol $\text{cell}^{-1} \text{h}^{-1}$	14.7	15.0	58.7	6.5
Sp- β -glc activity	femto mol $\text{cell}^{-1} \text{h}^{-1}$	12.4	9.1	29.4	2.1

TBA: Total bacterial abundance; **PAB:** Particle associated bacterial abundance; **TBp:** Total bacterial production; **PABp:** Particle-associated bacterial production; **SBp:** Specific bacterial production; **α -glc:** α -glucosidase activity; **β -glc:** β -glucosidase activity; **PF-glc activity:** Particle free glc activity; **Sp-glc activity:** Specific glc activity

TBp in the macroaggregates ranged from $2.3 \mu\text{g-C l}^{-1} \text{h}^{-1}$ (July '99) to $31.0 \mu\text{g-C l}^{-1} \text{h}^{-1}$ (January 2000) (**Fig. 2B.3b**). The TBp increased from $2.3 \mu\text{g-C l}^{-1} \text{h}^{-1}$ (July '99) to $28.8 \mu\text{g-C l}^{-1} \text{h}^{-1}$ (September '99) and declined to $4 \mu\text{g-C l}^{-1} \text{h}^{-1}$ (November '99). Thereafter, a maximal value was observed in January 2000 ($3.2 \mu\text{g-C l}^{-1} \text{h}^{-1}$), declined in February 2000 ($4 \mu\text{g-C l}^{-1} \text{h}^{-1}$) followed by a steady increase until the end of the sampling period. The values were generally $< 20 \mu\text{g-C l}^{-1} \text{h}^{-1}$ except for those recorded in the months of September 1999 and January 2000 (**Fig. 2B.3b**). Moreover, specific production in the macroaggregates ranged from

2.7 femto g-C cell⁻¹h⁻¹ (July '99) to 81.8 femto g-C cell⁻¹ h⁻¹ (April 2000) (**Table 2B.2**).

Table 2B.2: Mean, Sd and range of bacterial abundance, production, ecto-glucosidase activity, specific bacterial production, specific enzyme activity and enzyme ratio in the macroaggregate samples during 1999-2000.

Parameters		Mean	SD	Max	Minimum
TBA	x 10 ⁶ cells ml ⁻¹	1.4	0.8	2.9	0.5
PAB	x 10 ⁶ cells ml ⁻¹	0.9	0.7	2.2	0.1
TBp	µg-C l ⁻¹ h ⁻¹	12.2	9.2	31.0	2.3
PABp	µg-C l ⁻¹ h ⁻¹	9.1	7.1	28.0	2.1
SBp	femto g-C cell ⁻¹ h ⁻¹	33.5	23.7	81.8	2.7
Total α-glc	nM-C h ⁻¹	35.1	36.3	116.0	4.6
PA- α-glc	nM-C h ⁻¹	33.0	36.7	114.1	1.1
PF- α-glc	nM-C h ⁻¹	2.1	4.1	15.4	0.0
Total β-glc	nM-C h ⁻¹	31.5	35.7	126.1	1.5
PA- β-glc	nM-C h ⁻¹	28.9	36.4	123.8	2.6
PF- β-glc	nM-C h ⁻¹	1.0	1.9	6.5	0.0
α/β ratio		1.2	0.3	1.8	0.7
SP-α-glc activity	femto mol cell ⁻¹ h ⁻¹	101.4	93.7	270	20.0
SP-β-glc activity	femto mol cell ⁻¹ h ⁻¹	87.1	87.1	293	11.5

TBA: Total bacterial abundance; **PAB:** Particle associated bacterial abundance; **TBp:** Total bacterial production; **PABp:** Particle-associated bacterial production; **SBp:** Specific bacterial production; **α-glc:** α-glucosidase activity; **β-glc:** β-glucosidase activity; **PF-activity:** Particle free glc activity; **Sp-glc activity:** Specific glc activity

The particle free bacterial production (PFBp) values in the seawater samples varied from 0.1 µg-C l⁻¹ h⁻¹ (December '99) to 1.0 µg-C l⁻¹ h⁻¹ (April 2000) (**Fig. 2B.3c**). On the other hand, PFBp in macroaggregates ranged from 0.2 µg-C l⁻¹ h⁻¹ (July '99) to 18 µg-C l⁻¹ h⁻¹ (January 2000) (**Fig. 2B.3d**). PFBp in both seawater and macroaggregate samples showed small-scale variations.

The trend in seasonal variation of particle associated bacterial production (PABp) was relatively different from TBp in seawater samples. PABp values ranged from $0.2 \mu\text{g-C l}^{-1}\text{h}^{-1}$ (March 2000) to $3.1 \mu\text{g-C l}^{-1}\text{h}^{-1}$ (June 2000) (**Fig. 2B.3e**). The average PABp was $1.2 \pm 1 \mu\text{g-C l}^{-1}\text{h}^{-1}$, which accounted for 75% of the total bacterial production. During 1999, the PABp was lower between June to September despite higher TBp. Thereafter, PABp showed relatively higher values between October to January and later in the months of April and June 2000. On the other hand, the seasonality observed in PABp of macroaggregates was similar to that of TBp (**Fig 2B.3f**). The PABp values ranged from $2.1 \mu\text{g-C l}^{-1} \text{h}^{-1}$ (July '99) to $28 \mu\text{g-C l}^{-1} \text{h}^{-1}$ (September '99) and the average PABp was $9.1 \pm 7.1 \mu\text{g-C l}^{-1} \text{h}^{-1}$, constituted 75% of the total bacterial production, which was similar to the PABp in seawater samples.

2B.3.4 Ecto-glucosidase activity

The total α -glc recorded in the seawater samples during 1999-2000 ranged from 2.3 nM-C h^{-1} (March 2000) to $127.5 \text{ nM-C h}^{-1}$ (September '99) (**Fig. 2B.4a**). The α -glc activity increased from 19.5 nM-C h^{-1} (July '99) to $127.5 \text{ nM-C h}^{-1}$ (September '99), followed by a decline to 2.3 nM-C h^{-1} (March 2000). Thereafter, the activity increased steadily to reach 29.4 nM-C h^{-1} (July 2000). The specific α -glc activity ranged from $6.5 \text{ femto mol-C cell}^{-1} \text{ h}^{-1}$ (August '99) to $58.7 \text{ femto mol-C cell}^{-1} \text{ h}^{-1}$ (September '99) (**Table 2B.1**). The α -glc activity in the macroaggregate samples ranged from 4.6 nM-C h^{-1} (February 2000) to 116 nM-C h^{-1}

h^{-1} (September '99) (**Fig. 2B.4b**). The α -glc activity of the macroaggregates had higher values during September '99 (116 nM-C h^{-1}) and May 2000 (107.7 nM-C h^{-1}) and was lower during November to February (1.1 nM-C h^{-1} ; February 2000). The specific α -glc activity ranged from 20 femto $\text{mol-C cell}^{-1} \text{h}^{-1}$ (February 2000) to 270 femto $\text{mol-C cell}^{-1} \text{h}^{-1}$ (September '99) (**Table 2B.2**).

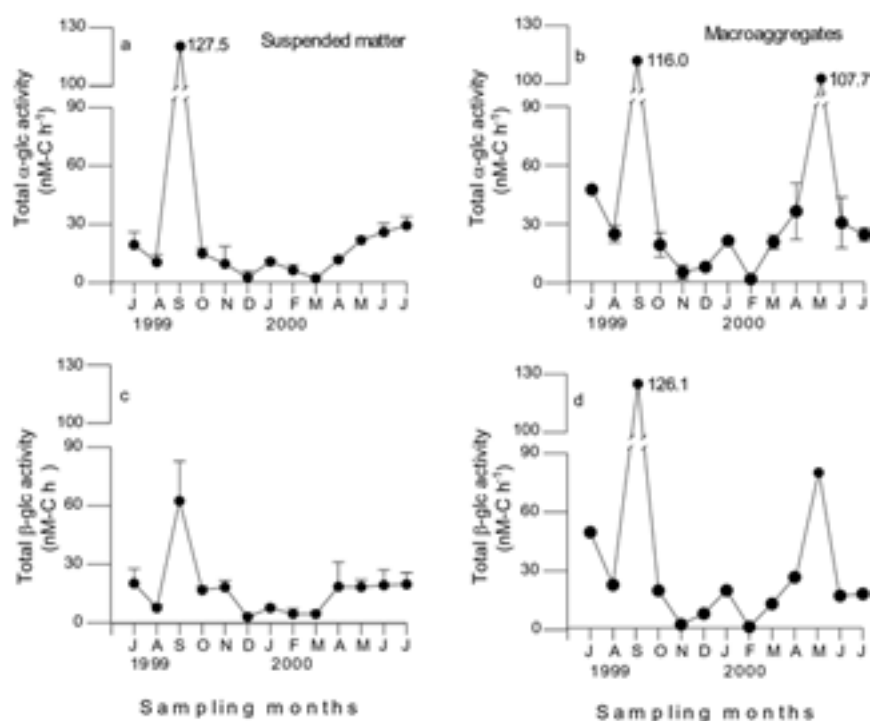


Fig. 2B.4 Seasonal variation of Total α -glc and Total β -glc activity in the suspended matter (a & c) and macroaggregates (b & d) in the Dona Paula Bay during 1999-2000.

The total β -glc activity in the seawater samples during the study period ranged from 3 nM-C h^{-1} (December '99) to 62.4 nM-C h^{-1} (September '99) (**Fig. 2B.4c**). The β -glc activity declined from 20.2 nM-C h^{-1} (July '99) to 7.7 nM-C h^{-1} , followed

by an increase to 62.4 nM-C h^{-1} (September '99). Thereafter, the activity decreased to 3 nM-C h^{-1} (December '99), increased to 18.5 nM-C h^{-1} during April 2000 and did not vary further. The specific β -glc activity ranged from $2.1 \text{ femto mol-C cell}^{-1} \text{ h}^{-1}$ (August '99) to $29.4 \text{ femto mol-C cell}^{-1} \text{ h}^{-1}$ (September '99) (**Table 2B.1**). The total β -glc activity and its seasonality in the macroaggregate samples were similar to that of α -glc activity of macroaggregates and ranged from 1.5 nM-C h^{-1} (February 2000) to $126.1 \text{ nM-C h}^{-1}$ (September '99) (**Fig. 2B.4d**). The specific β -glc activity in the macroaggregates ranged from $11.5 \text{ femto mol-C cell}^{-1} \text{ h}^{-1}$ (November 2000) to $293 \text{ femto mol-C cell}^{-1} \text{ h}^{-1}$ (May 2000) (**Table 2B.2**).

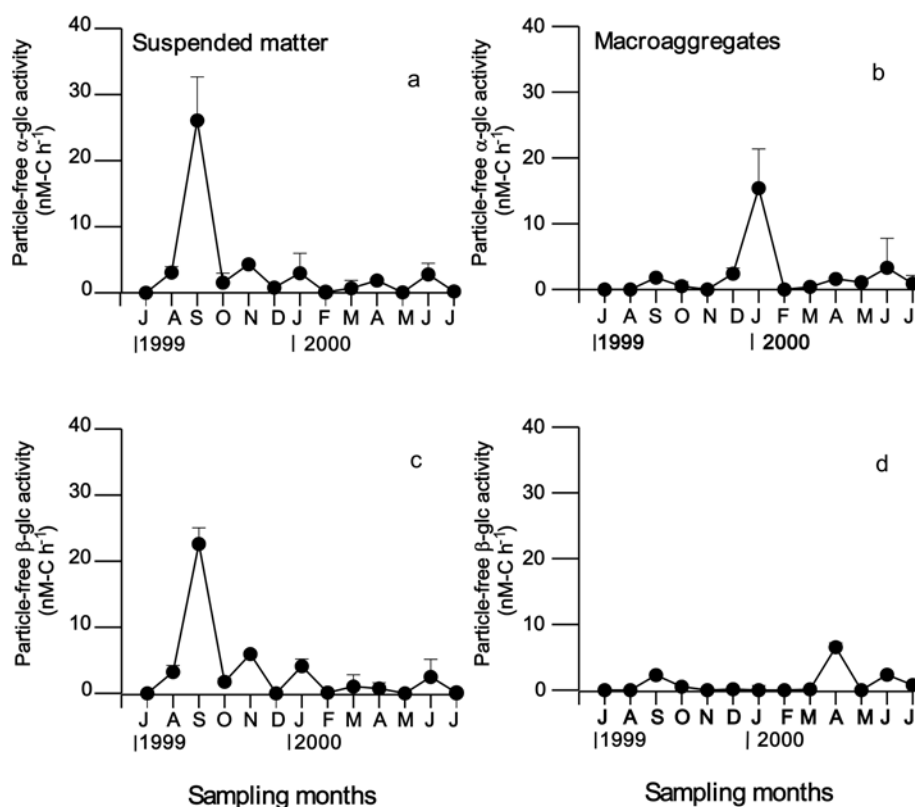


Table 2B.5 Seasonal variation of Particle-free α -glc and Particle-free β -glc activity in the suspended matter (a & c) and macroaggregates (b & d) in the Dona Paula Bay during 1999-2000.

Except for the high enzyme activity recorded for both particle-free (PF) α -glc (26.1 nM-C h⁻¹) (**Fig. 2B.5a**) and PF β -glc activity (22.6 nM-C h⁻¹) (**Fig. 2B.5c**) in the month of September '99, the seasonal variation of the PF-glc activity in the suspended matter did not have any specific trend and showed small-scale variations. The mean PF- α glc and PF- β glc activity for the entire study period was 3.4 ± 7.2 (16.4% of total α -glc) and 4.2 ± 6.7 nM-C h⁻¹ (25% of total β -glc), respectively (**Table 2B.1**). Similarly, except for a high of 15.4 nM-C h⁻¹ (January 2000) of PF α -glc activity (**Fig. 2B.5c**) and 6.3 nM-C h⁻¹ (April 2000) of β -glc activity in the macroaggregate samples, the samples showed little PF-glc activity (**Fig. 2B.5d**), respectively. The average PF- α -glc and PF- β -glc activity in the macroaggregates for the entire study period was 2.1 ± 4.1 (6% of total α -glc activity) and 0.98 ± 1.9 nM-C h⁻¹ (3.1% of total β -glc activity), respectively.

The particle-associated (PA) glc-activity (α - or β -glc) was calculated as the difference between the total glc and PF-glc activity and constituted a significant fraction of the total glucosidase activity. The temporal variations of PA-glc activity was similar to that of total glc activity in both seawater and macroaggregate samples. The PA- α and PA- β glc activity ranged from 1.6 nM-C h⁻¹ (March 2000) to 101.4 nM-C h⁻¹ (September '99) and 3 nM-C h⁻¹ (December '99) to 39.8 nM-C h⁻¹ (September '99), respectively (**Fig. 2B.6a & 2B.6b**). The mean PA- α and β -glc activity in the seawater was 19.2 ± 26.2 nM-C h⁻¹ (85% of total α -glc activity) and 13.8 ± 10.4 nM-C h⁻¹ (81% of total β -glc activity), respectively (**Table 2B.1**). The PA- α - and β -glc activity of macroaggregates ranged from 1.1 nM-C h⁻¹

(February 2000) to 114.1 nM h^{-1} (September '99) and below detectable limits to $123.8 \text{ nM-C h}^{-1}$ (September '99), respectively (**Fig. 2B.6c & 2B.6d**). The mean PA- α - and β -glc activity of macroaggregates was $33 \pm 36.7 \text{ nM-C h}^{-1}$ (94% of total α -glc activity) and $28.9 \pm 36.4 \text{ nM-C h}^{-1}$ (92% of total β -glc activity), respectively (**Table 2B.2**). The extracellular (dissolved) glucosidase activity was negligible.

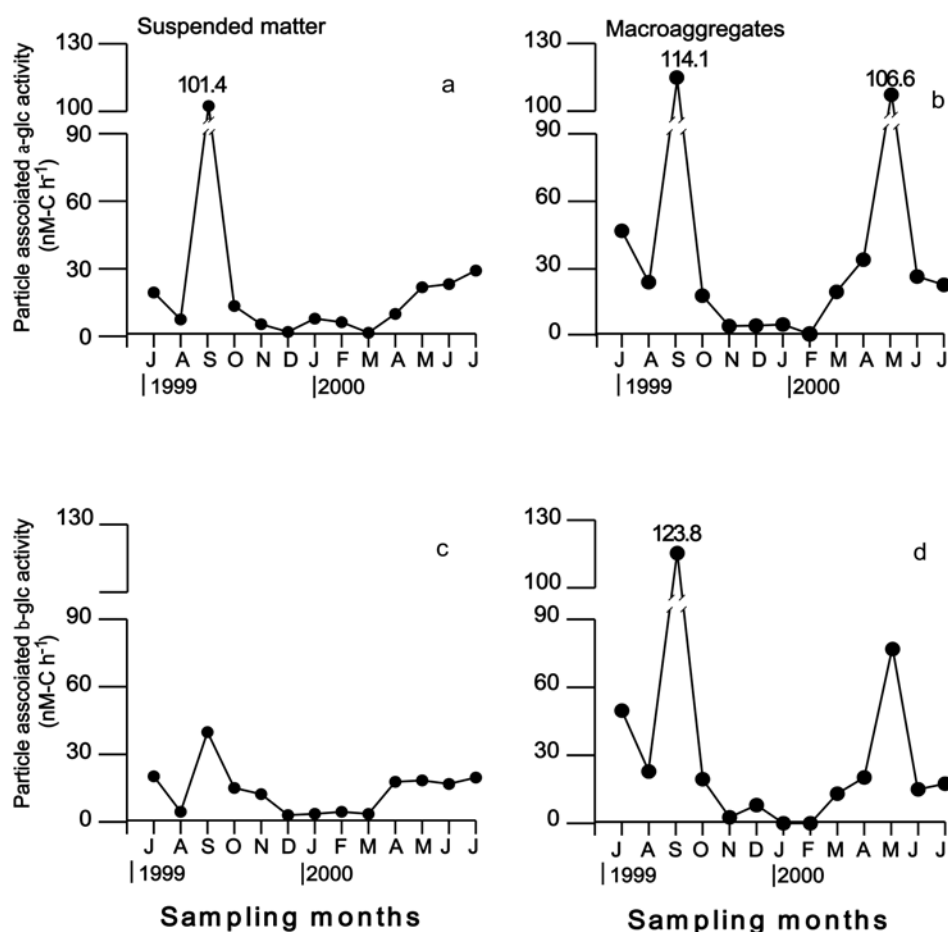


Fig. 2B.6 Seasonal variation of Particle-associated α -glc and Particle associated β -glc activity in the suspended matter (a & c) and macroaggregates (b & d) in the Dona Paula Bay during 1999-2000.

2B.3.5 α -glc/ β -glc ratios

The ratios in seawater and macroaggregate samples varied from 0.5 (March 2000) to 2.0 (September '99) and 0.7 (February 2000) to 1.8 (November '99), respectively (**Fig. 2B.7**). The average enzyme ratio in seawater (1.1 ± 0.4) and macroaggregate (1.2 ± 0.3) was similar (**Table 2B.1 & 2B.2**). Interestingly, the ratio in seawater samples showed a trend opposite to that observed for macroaggregates (**Fig. 2B.7**).

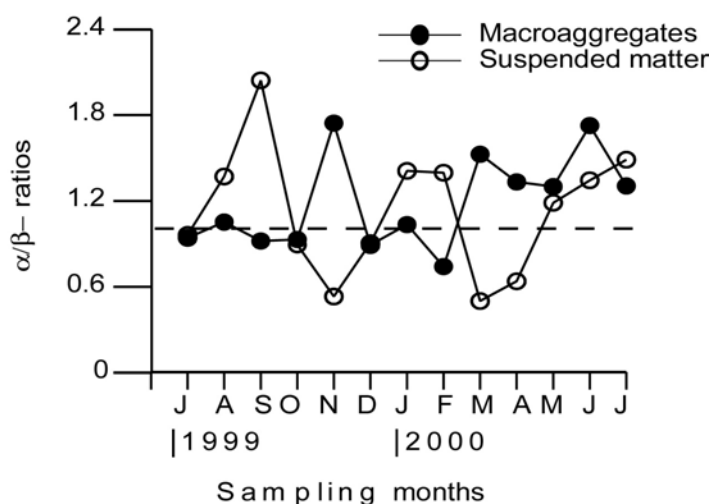


Fig. 2B.7. Changes in the α/β ratio of the enzyme activity in both suspended matter (-O-) and macroaggregates (-●-) from the Dona Paula Bay during 1999-2000.

2B.3.6 Statistical Analyses

Bacterial production and enzyme activity in seawater and macroaggregates had no significant correlation with hydrography or nutrients (**Table 2B.3 & 2B.4**). In

the seawater samples, the TBp had a weak linear relationship with Chl *a* ($r=0.513$, $p<0.07$) (**Table 2B.3**), whereas PBp had a significant linear correlation with Chl *a* ($r=0.625$; $p<0.01$). However, TBp had no significant correlation with EPS (NaCl-soluble or EDTA-soluble) and TEP (**Table 2B.3**). Similarly, bacterial enzyme activity too did not have any correlation with any of the bulk particulate parameters. Bacterial production and enzyme activity in the macroaggregates had insignificant relationship with Chl *a*, EPS (NaCl-soluble and EDTA-soluble) and TEP (**Table 2B.4**). However, highly significant correlation was recorded between total bacterial production and enzyme activity in seawater samples only (**Tables 2B.3**).

The influence of seawater samples on the microbial activity in macroaggregates was also assessed. Bacterial production, abundance and extracellular enzyme activity in macroaggregate samples were correlated with suspended load, Chl *a*, EPS and TEP concentrations in the seawater samples (Table 2B.5). The EDTA-soluble EPS of the seawater samples had significant correlation with total α -glc activity ($r=0.639$; $p<0.001$) and weak correlation with β -glc activity ($r=0.539$; $p<0.06$). Similarly, Chl *a* concentrations of water column had linear trends with bacterial production ($r=0.434$) and total glc activity (**Table 2B.5**).

Table 2B.3: Pearsons correlation coefficients of bacterial production, enzyme activity and enzyme ratio with various particulate parameters of the seawater samples during 1999-2000 in the Dona Paula Bay.

Parameters	TBp	Total α -glcA	PA- α -glcA	Total β -glcA	PA- β -glcA	α/β ratio
TBA	-0.059	-0.310	-0.332	-0.371	-0.425	-0.215
TBp		0.677**	0.684**	0.643**	0.624**	0.519
Total α -glcA			0.996***	0.959***	0.865***	0.698**
PA- α -glcA				0.959***	0.889***	0.714**
Total β -glcA					0.954***	0.544*
Suspended load	0.208	-0.250	-0.172	-0.180	0.084	-0.047
Chl a	0.513	0.349	0.358	0.360	0.382	0.352
POC	0.003	-0.070	-0.070	0.014	0.096	-0.204
TEP	0.289	-0.018	-0.018	-0.010	-0.004	-0.179
NaCl-soluble EPS	0.152	0.102	0.090	0.305	0.361	-0.198
EDTA-soluble EPS	-0.167	0.220	0.259	0.223	0.306	0.329

*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Table 2B.4: Pearsons correlation coefficient for bacterial production, enzyme activity and enzyme ratio with various particulate parameters of the macroaggregate samples during 1999-2000 in the Dona Paula Bay.

Parameters	TBp	Total α -glcA	PA- α -glcA	Total β -glcA	PA- β -glcA	α/β ratio
TBA	-0.136	0.370	0.384	0.408	0.445	-0.227
TBp		0.316	0.230	0.383	0.273	-0.190
Total α -glcA			0.994***	0.972***	0.963***	-0.078
PA- α -glcA				0.965***	0.972***	-0.067
Total β -glcA					0.988***	-0.213
Total macroaggregate	0.372	0.239	0.256	0.190	0.206	-0.10
Chl <i>a</i>	0.075	0.497	0.481	0.391	0.383	0.03
POC	-0.173	0.138	0.131	-0.008	-0.004	0.230
TEP	-0.076	-0.006	-0.001	-0.079	-0.080	0.349
NaCl-soluble EPS	-0.124	0.076	0.087	0.114	0.152	-0.230
EDTA-soluble EPS	-0.389	0.186	0.217	0.111	0.171	0.321

*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Principal component analyses (PCA) of both seawater and macroaggregate samples was carried out to further assess the effect of various particulate parameters on the bacterial production and enzyme activity. The data were subjected to Varimax rotation and two factors were extracted from seawater data and three factors from macroaggregate data (**Tables 2B.6 & 2B.7**). The factor loadings of seawater samples and macroaggregates explained 84% and 71% of the data, respectively. When the factor loadings of seawater samples were

plotted against each other (**Fig. 2B.8a**), TEP, NaCl-soluble EPS and POC formed one group whereas TBp, enzyme activity, Chl *a* and EDTA-soluble EPS formed another group. Both suspended load and total bacterial count formed remained independent. Similarly, EPS (NaCl-soluble EPS and EDTA-soluble EPS), total macroaggregates and bacterial abundance in macroaggregates formed one group whereas enzyme activity, bacterial production and Chl *a* formed the other group (**Fig. 2B.8b**). TEP and POC remained independent of all the other parameters in macroaggregate samples.

Table 2B.5: Pearsons correlation of bacterial abundance, production and enzyme activity of macroaggregates with particulate parameters of suspended particulate matter.

Parameters	Suspended load	Chl <i>a</i>	POC	NaCl-soluble EPS	EDTA-soluble EPS	TEP
TBA	0.578*	-0.414	0.02	0.241	0.372	-0.422
TBp	-0.256	0.434	-0.318	-0.174	-0.168	0.197
Total α -glc	0.161	0.395	0.272	0.030	0.639**	-0.181
PA- α -glc	0.166	0.367	0.301	0.054	0.663**	-0.188
Total β -glc	0.042	0.307	0.202	0.073	0.539*	-0.256
PA- β -glc	0.058	0.263	0.213	0.083	0.580*	-0.260

*= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$.

Table 2B.6. Factor loadings and eigen values obtained from various particulate parameters of the suspended matter after performing principal component analyses.

Parameters	Factor 1	Factor 2
Dry weight	-0.072	-0.949
Chl <i>a</i>	0.667	0.624
POC	0.810	0.544
EDTA-EPS	0.820	0.461
NaCl-EPS	0.696	0.614
TEP	0.545	0.618
Bacterial abundance	0.087	0.825
Bacterial production	0.853	0.480
Total- α -glc A	0.970	0.214
Total- β -glc A	0.937	0.146
Eigen values	7.642	1.616
Variance	5.711	3.547
Total	0.519	0.322

Table 2B.7. Factor loadings and eigen values obtained from various particulate parameters of the macroaggregates after performing principal component analyses.

Parameters	Factor 1	Factor 2	Factor 3
Dry weight	0.703	0.018	0.664
Chl <i>a</i>	0.050	0.590	-0.225
POC	0.077	-0.007	0.837
EDTA-EPS	0.796	0.011	-0.014
NaCl-EPS	0.764	-0.003	-0.046
TEP	-0.503	-0.094	0.605
Bacterial abundance	0.885	0.245	0.083
Bacterial production	-0.384	0.546	-0.264
Total- α -glc A	0.156	0.947	0.208
Total- β -glc A	0.161	0.941	0.097
Eigen values	3.407	2.512	2.094
Variance	2.954	2.497	1.690
Total	0.295	0.250	0.169

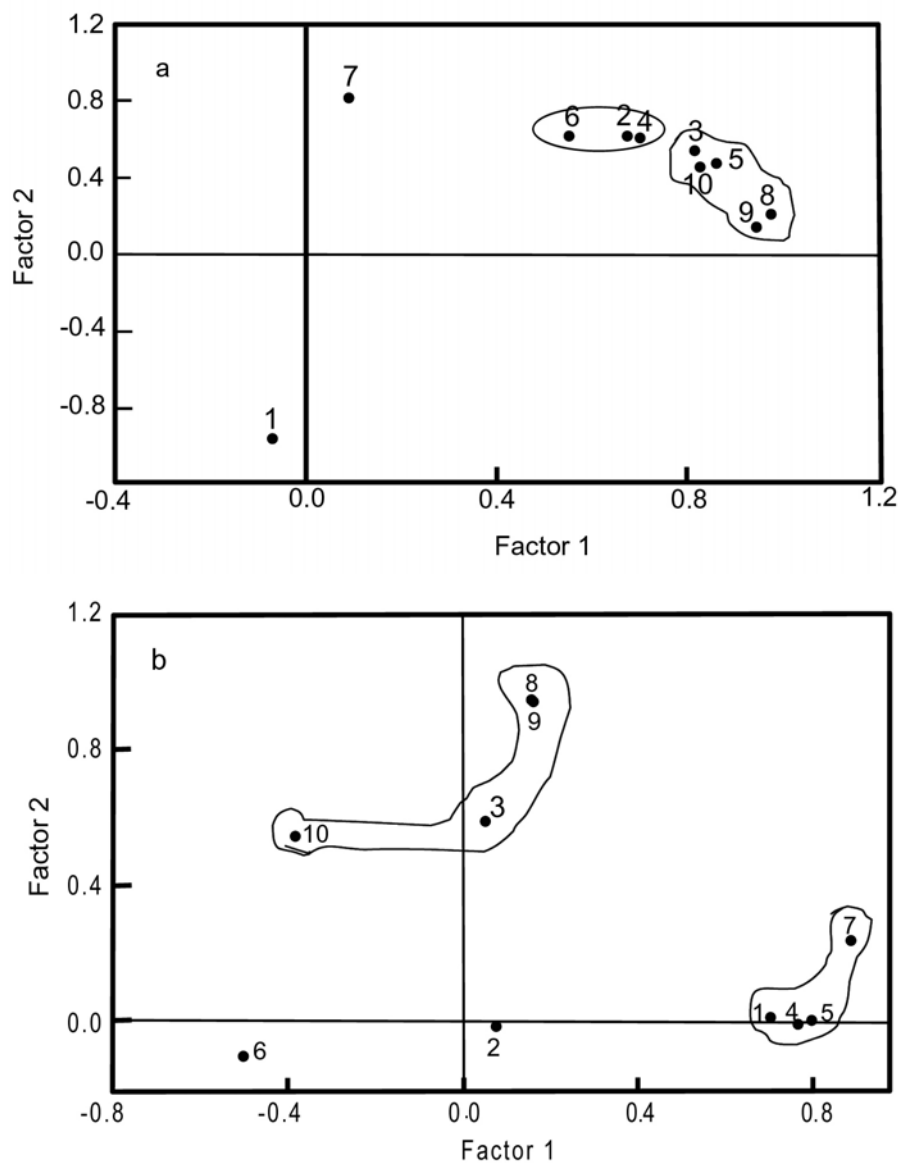


Fig. 2B.8 Varimax normalized PCA plots of various particulate parameters of seawater samples (a) and macroaggregate samples (b) collected from Dona Paula Bay. Each number represents a particulate parameter as shown below:

1: Suspended load or Total macroaggregates; 2: POC; 3: Chl a; 4: NaCl-soluble EPS; 5: EDTA-soluble EPS; 6: TEP; 7: TBA; 8: Total α -glc; 9: Total β -glc and 10: TBp.

2B.4 Discussion

2B.4.1 Ecto-glucosidase activity

The estimates of enzyme hydrolysis rates are influenced by the substrate concentration, incubation period and competitive inhibition of the substrate analogs by naturally occurring organic substrates (Hoppe 1983). In the present study, samples were inoculated with lower MUF-substrate concentrations and variable incubation time. Although 2 h incubation was optimum (**Fig. 2B.1a**), certain samples (especially macroaggregates) were incubated longer to ensure adequate hydrolysis. Lower concentration of MUF-substrate was preferred (**Fig. 2B.1b**) to minimise the interference from water column turbidity (Somville 1988). Monsoon influenced seasonal variation in α - and β -glc activity was observed in both seawater samples and macroaggregate samples. The total α - and β -glc activity in the water column were in the range of 2.3 to 128 nM-C h⁻¹ and 3 to 62.4 nM-C h⁻¹, respectively (**Table 2B.1**). Moreover, α - and β -glc activity showed similar seasonal behavior with exceptionally high activity during late monsoon (September '99) in the water column and macroaggregates (**Figs. 2B.4a - d**). There are not many studies on heterotrophic enzyme activity in tropical coastal waters (Rath et al 1993). Moreover, no information is available on the seasonal variations of bacterial heterotrophy in the coastal waters of India. Hence, comparison with other tropical data was not possible. The range of heterotrophic enzyme activities at the study site were similar to those reported for a mesotidal

estuary (Cunha et al 2001; β -glc activity- 4.3 to 181.3 nM h⁻¹) but higher than those reported in a tropical coastal waters (Rath et al 1993; α -glc- 0.3 to 1.6 nM h⁻¹ and β -glc- 0.3 to 1.8 nM h⁻¹), eutrophic lake (Middleboe and Søndergaard 1993; β -glc- 7.8 to 22 nM h⁻¹) and temperate coastal waters (Nausch et al 1998; α -glc- 0.5 to 18 nM h⁻¹ and β -glc- 0.8 to 12 nM h⁻¹). However, most of these works were carried out in temperate waters and hence direct comparisons are only subjective.

In seawater samples, extracellular enzyme activity did not show any correlation with other bulk particulate parameters like dry weight, POC and NaCl-soluble EPS (**Table 2B.4**). On the other hand, Chl *a* and EDTA-soluble EPS had a linear trend with the enzyme activity. This is also reflected in the PCA plots (**Fig. 2B.8a**) as the enzyme activity is grouped with Chl *a* and EDTA-soluble EPS. On the other hand, enzyme activity in macroaggregates had positive trend with Chl *a* concentrations and no correlation with EPS and TEP (**Table 2B.5**), which is further substantiated in the PCA plots (**Fig. 2B.8b**) where the enzyme activity is grouped with Chl *a*. This suggests that although statistically significant correlations were not observed, Chl *a* and to some extent EDTA-soluble EPS appeared to influence microbial enzyme activity in both water column macroaggregate samples. The enzyme activity of macroaggregate samples showed moderate to significant correlation with EDTA-soluble EPS of seawater samples. From the correlations it appears that the composition of organic matter in the water column might also influence the macroaggregate associated

bacterial enzyme activity. On the other hand, factors like substrate quality, terrestrial inputs and river-runoff might also be important in regulating the bacterial activity in the study area.

The distribution of the two forms of glc enzymes have been widely used to assess the quality of the POM accumulating in the water column. It has been suggested that α -glc activities are higher when the aggregates/particulate organic matter is freshly derived from phytoplankton and organic rich. On the other hand, higher β -glc activity highlights the accumulation of aged and decomposed aggregates in the water column (Decho 1995, Karen and Rassoulzadegan 1995, Decho and Herndl 1995). Hence the ratio of α -glc/ β -glc was used to explain the quality of the particulate organic matter in the study area. The average α -glc/ β -glc ratio of the seawater samples was not very different from macroaggregates (**Tables 2B.1 & 2B.2**). However, the seasonal variations of the ratios indicate that the changes in the composition of macroaggregates were inverse to that of suspended particles during most of the sampling period (**Fig. 2B.8**). For example, phytoplankton blooms during September '99 (**Fig. 2A.6a; Chapter 2A**) appeared to influence the α -glc/ β -glc ratios in the surface waters suggesting that blooms were one of the major sources of labile organic matter in the seawater samples (**Fig. 2B.8**). In contrast to the seawater samples, the ratios of the macroaggregate sample was lower during September '99. Similarly, the α / β -ratios of macroaggregates were 3-folds greater than that of seawater samples during March 2000 to April 2000 although no major blooms were observed during

this period. One reason for such changes in particle composition could be a shift in the composition of phytoplankton grazing community. Earlier reports from the study area have shown that the composition of the grazing community shifts from herbivores in post-monsoon (October to January) months to carnivores in pre-monsoon (February to May) (Gauns 2000). This might lead to increased contribution of detritus and fecal matter to the total macroaggregates during post-monsoon (October to January) and increased contribution of phytoplankton to the macroaggregates in the pre-monsoon months (February to May). Such a shift in grazing behavior changes the composition of the aggregates, which is also seen in the microbial activity.

2A.4.2 Bacterial production and abundance

Bacterial production is indicative of metabolically active cells and showed distinct seasonality during the study period ranging from 0.51 to 3.6 $\mu\text{g-C l}^{-1} \text{ h}^{-1}$ in the water column and 0.013 to 5.15 $\text{ng-C mg}^{-1} \text{ aggregate h}^{-1}$ (Table 1). The average bacterial production (0.39 to 3.6 $\mu\text{g-C l}^{-1} \text{ h}^{-1}$) in the surface waters was higher than those previously reported in these waters (0.3 to 1.6 $\mu\text{g-C l}^{-1} \text{ h}^{-1}$; Gomes et al 1991) but similar to those reported by Ram et al (2002) (0.6 to 6 $\mu\text{g-C l}^{-1} \text{ h}^{-1}$) in these waters. The TBp values recorded in these waters were similar to those reported for a tidal temperate estuary (0.49 to 5.3 $\mu\text{g-C l}^{-1} \text{ h}^{-1}$, Cunha et al 2000) but were much lower than those reported in near-shore tropical waters (9.2 to 15.5 $\mu\text{g-C l}^{-1} \text{ h}^{-1}$; Rath et al 1993) and estuarine waters (0.5 to 10 $\mu\text{g-C l}^{-1} \text{ h}^{-1}$;

Roland and Cole 1999). Moreover, the mean bacterial abundance in the water column was $2.4 \pm 2 \times 10^6$ cells ml⁻¹ which was similar to those reported by Ram et al (2002) (1.6 to 5×10^6 cells ml⁻¹) but higher than those reported by Devassy and Goes (1989) (0.203 to 0.405×10^3 cells ml⁻¹) and Gauns (2000) (0.043 to 0.36×10^5 ml⁻¹) in the Dona Paula Bay. However, these counts were lesser than those reported from the eastern coast of India (Ramaiah et al 1995) (10^7 to 10^8 cells ml⁻¹). The bacterial abundance at the study site was similar to those reported from a temperate estuary (2.8 to 3.5×10^6 cells ml⁻¹, Kirchman and Hoch 1988) but less than those reported from a mesotidal estuary (2.6 to 14×10^6 cells ml⁻¹, Cunha et al 2000).

In an estuarine environment, changes in the quality and quantity of organic matter and growth conditions can alter bacterial number, activity and community structure. During October to January, higher total bacterial production and abundance was recorded in both seawater samples (**Figs. 2B.2a & 2B.3a**) and macroaggregates (**Figs. 2B.2b & 2B.3b**). These higher values coincided with phytoplankton blooms in the water column (**Fig. 2A.6a; Chapter 2A**). Similarly, higher Chl a concentrations in the month of June 2000 induced a similar bacterial response in TBp and total enzyme activity. The phytoplankton blooms during September-October '99 and June 2000 were dominated by diatoms like *Skeletonema*, *Nitzschia* and *Fragillaria* sp., which produce EPS during their growth. Bacteria actively degrade exudates of diatoms to meet their organic carbon requirement (Goto et al 2001). It is possible that release of these

compounds might have increased the microbial activity as evident from the high bacterial production and glucosidase activity (**Figs. 2B.3a and 2B.4a**).

Particle colonizing bacteria in aquatic environments form 5-10 % of total bacterial population in open oceans (Alldredge and Gotschalk 1990, Turley and Stutt 2000) and up to 90% of the total bacterial population in coastal and riverine ecosystems (Bell and Albright 1981, Crump and Baross 2000). In the present study, the percent of attached bacteria (3 to 79%) was similar to those reported earlier in these waters (4 to 98%, DeSouza 2000) but significantly higher than those reported for oligotrophic coastal waters (17%, Iriberry et al 1990), riverine waters (9.5%, Berger et al 1996) and in temperate lakes (11%, Friedrich et al, 1999). This was also reflected in the particle associated bacterial production (PA-Bp) that formed a major fraction (up to 75%) of the total bacterial production. Moreover, a major fraction of α - and β -glc enzyme activity (85% and 81 %, respectively) was also associated with particles and was significantly correlated with both particle associated bacterial production and abundance (**Tables 2B.3 & 2B.4**). This indicates that much of the enzymatic transformation of the organic matter in the water column was tightly coupled with production and bacterial abundance associated with the particles. It has been reported that bacteria growing on particles (seawater samples or macroaggregates) have higher growth efficiency, are bigger and hydrolyze and convert POM to DOM at a faster rate (Smith et al 1992, Decho and Herndl 1995, Grossart and Ploug 2000, Simon et al 2002). In contrast, bacterial production rates in macroaggregates have been

found generally to be same or less than in the surrounding waters (Kaltenbock and Herndl 1992, Muller-Niklas et al 1994, Simon et al 2002). In this study, the PABp was generally > the PFBp in both seawater samples and sinking macroaggregates. Thus, it appears that the organic matter broken down and released by particle-associated bacteria as dissolved organic matter (DOM) might not be sufficient to support the particle free bacteria. On the other hand, low PFBp might probably be due to less reactive DOM in the water column. In an earlier study, Wafar et al (1997) had suggested the importance of organic carbon derived by the dissolution of POM and its flux in these waters. The high particle associated bacterial production and heterotrophic activity recorded in this study might play an important role in sustaining the DOM levels in the study area.

The characteristics of macroaggregates are distinctly different from suspended particles. Although both contain organic matter from diverse sources and forms, macroaggregates are generally rich in detritus and fecal matter except during the course of a phytoplankton bloom wherein large number of intact phytoplankton cells is also found in the macroaggregates (Deuser et al 1981, Martin et al 1987). Sediment trap studies have shown that sinking particles are rich in organic carbon compared to suspended particulate matter (Falkowski et al 1994, Herndl 1995). Moreover, microbial activity (production, respiration and enzyme activity) in sinking aggregates like marine snow may exceed that of the surrounding waters (Karner and Herndl 1992, Grossart and Simon 1988, Unanue et al 1998). In the present study, the specific bacterial production associated with

macroaggregates were 28-folds greater than those in the seawater samples (**Tables 2B.1 & 2B.2**). Similarly, specific enzyme activity was 7-folds higher in macroaggregate samples as compared to seawater samples (**Tables 2A.1 & 2A.2**). Such higher cell-specific production and enzyme activity indicates the macroaggregate-associated bacteria actively mineralized labile form of organic matter from the macroaggregates. Moreover, it also points that the physiological state of the bacterial community associated with these particles were different from those in the surrounding waters. The sinking aggregates appeared to be hot spots of bacterial activity and remineralization compared to other forms of particulate matter.

2B.5 Conclusions

Temporal variations in bacterial abundance and production, enzyme activity and enzyme ratios were observed in both seawater samples and macroaggregates in the Dona Paula Bay. Seasonal changes in seawater samples and macroaggregate composition influenced the microbial activity at the study area. Most of the microbial activity was associated with particulate matter in both seawater and macroaggregate samples. Particle-associated bacteria therefore appeared to play a major role in organic matter transformations. Both phytoplankton biomass and EDTA-soluble EPS appeared to influence the bacterial enzyme activity in the seawater and macroaggregate samples. Although primary production is the major source of organic carbon in these waters, inputs

from POM including EPS and its turnover in the water column might be equally important in sustaining the microbial carbon demand.