

## **Monsoon induced changes in the size fractionated phytoplankton biomass and production rate in the estuarine and coastal waters of southwest coast of India**

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### **Abstract**

Changes in the autotrophic pico- (0.2-2 $\mu$ m), nano- (2-20 $\mu$ m) and micro- (>20 $\mu$ m) plankton biomass (chlorophyll *a*) and primary production were measured in the estuarine and coastal waters off Cochin, southwest coast of India during the onset and establishment of a monsoon. During this period, the estuary was dominated by nutrient rich fresh water, whereas the coastal waters were characterized with higher salinity values (>30 psu) and less nutrients. The average surface chlorophyll *a* concentrations and primary production rates were higher in the estuary (av. 13.7 mgm<sup>-3</sup> & 432 mgC m<sup>-3</sup>d<sup>-1</sup>) as compared to the coastal waters (5.3 mgm<sup>-3</sup> & 224 mgC m<sup>-3</sup>d<sup>-1</sup>). The nanoplankton community formed the major fraction of chlorophyll *a* and primary production, both in the estuary (av. 85  $\pm$  SD 8.3% & av. 81.2  $\pm$  SD 3.2%) and the coastal waters (av. 73.2  $\pm$  SD 17.2% & av. 81.9  $\pm$  15.7%). Nanoplankton had the maximum photosynthetic efficiency in the coastal waters (av. 4.8 $\pm$  SD 3.9 mgC mgChl *a* m<sup>-3</sup> hr<sup>-1</sup>), whereas in the estuary, the microplankton had higher photosynthetic efficiency (av. 7.4  $\pm$  7 mgC mgChl *a* m<sup>-3</sup> hr<sup>-1</sup>). The heavy cloud cover and increased water column turbidity not only limit the growth of large-sized phytoplankton in the Cochin estuary and coastal waters, but also support the proliferation of nanoplankton community during the monsoon season, even though large variation in nanoplankton chlorophyll *a* and production exists between these two areas.

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Keywords - Phytoplankton; Size structure; Primary production; Nanoplankton; Cochin estuary.

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## **Introduction**

The size of planktonic organisms largely determines their interactions and hence, the rate of biological processes (Amstrong, 1994; Tang, 1995). The size structure of phytoplankton is very important as it changes with the environmental conditions (Malone 1980), reflecting the pathways of carbon cycling in the pelagic food chain (Legendre and Le Fevre, 1989). Typically, large sized phytoplankton have greater potential to export organic matter through a short, classical food chain, whereas the small-sized phytoplankton are utilized by complex microbial food webs that favour the recycling of organic matter (Cermeno et al, 2006). Many studies have confirmed that small sized phytoplankton is an integral component of the plankton community (Legendre and Le Fevre, 1989; Cermeno et al 2006), although, their relative contribution to the total community varies with the abundance of large sized phytoplankton (Raimbault et al., 1988; Chisholm, 1992). Generally nutrient enrichment favors the growth of large phytoplankton (Chisholm, 1992), while the production of small phytoplankton (nano-) are mainly controlled by microzooplankton (ciliates and flagellates) grazing (Riegman et al., 1993, Jyothibabu et al., 2006).

The Cochin estuary (9° 40' & 10° 12'N and 76° 10' & 76° 30'E) is a tropical micro-tidal estuary with two permanent openings to the Arabian Sea and sustains high biological production (Qasim, 2003). This tropical estuary is influenced by monsoon rainfall and associated run off which eventually result in changes in the overall functions of the trophic food web (Jyothibabu et al., 2006; Madhu et al., 2007). The annual rainfall in the Cochin estuary is about 3.2 m, which exhibits considerable variations from year to year. Approximately 75% of the total rainfall occurs during the monsoon period starts from late May or early June to September (Qasim 2003). With the onset of the monsoon and within the course of a few weeks the hydrographic conditions in the estuary undergoes remarkable changes due to the influx of fresh water as the system rapidly changes from marine to brackish water condition (Menon, et al., 2000). The present study examines changes in the environmental conditions and their responses on the distribution of different size fractions of phytoplankton (>20µm, 2-20µm and <2µm) in the coastal and estuarine waters during the monsoon months.

## **Materials and Methods**

Hydrographic observations were made once in a month (June, July, August and September 2006) from two stations, one in the Cochin estuary and the other in the coastal waters (Figure 1). The

sampling was carried out using a power boat and the water samples were collected from the surface and bottom (10 m depth at coastal station and 3 m depth in the estuary) using 5 litre Niskin bottles. The chemical parameters dissolved oxygen (DO), pH, inorganic ammonia (NH<sub>4</sub><sup>+</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), phosphate (PO<sub>4</sub><sup>-</sup>) and silicate (SiO<sub>3</sub><sup>-</sup>) were analyzed according to standard protocols (Grasshoff, 1983). Size fractionated phytoplankton pigments (chlorophyll *a*) were carried out by sequential filtering of 500ml water, initially through 20µm nylon sieve and subsequently through 2µm and 0.2µm polycarbonate filters. The cells retained by the 20µm sieve are the microplankton whereas those retained by 2 and 0.2µm filters constitutes the nano- and pico- fractions, respectively. After filtration, pigments were extracted in 90% acetone for 24h in the dark at 4°C and pigment concentration was determined spectrophotometrically (UV-VIS Spectrophotometer, Model - 1650PC, Shimadzu). Similarly, the size fractionated primary production was determined by running simulated '*in-situ*' incubations with the isotope <sup>14</sup>C. Four 300ml acid washed BOD glass bottles (3 light and 1 dark bottles) were filled with water collected from the surface and bottom. Each bottle was inoculated with 1ml 5µCi NaH<sup>14</sup>CO<sub>3</sub> and incubated for 6hours starting at noon. At the end of incubations, samples were sequentially filtered through 20µm nylon mesh and 2 and 0.2µm polycarbonate filters. The phytoplankton cells concentrated in the 20µm mesh were washed with filtered (through 0.2 polycarbonate filters) seawater and again concentrated on GF/F filters (nominal pore size - 0.7µm). The filters were used for subsequent analysis in the liquid scintillation counter after treatment with concentrated HCl fumes to remove inorganic carbon. The primary production rate was calculated according to the equation described in the protocol (UNESCO, 1994).

## Results

### *Environmental conditions*

During the entire observations, the estuarine waters remained warmer (av. >29°C) than the coastal waters (av. <28°C) both in the surface and bottom (Figs. 2a & b). Salinity showed wide variation between the coastal waters (av >30 *psu*) and estuary (<9 *psu*; Figs. 2e & f) pH was always high in the coastal waters (av.8.2) as compared to the estuary (av. 7.2; Fig. 2). Similarly, the coastal waters remained fairly saturated with respect to DO except during July and August, when the bottom waters became oxygen deficient (Figs. G & h). The dissolved nutrients such as DIN (the sum of inorganic ammonia, nitrite and nitrate), dissolved inorganic phosphorous (DIP) and dissolved inorganic silica (DISi) were also high in the estuary throughout the monsoon months as compared to the coastal

waters (Fig. 3). The inorganic nitrate ( $\text{NO}_3^-$ ) constituted the major fraction of the total DIN (av. >60%) in the surface and bottom waters of the estuary, whereas in the coastal waters, both  $\text{NO}_3^-$  and ammonia ( $\text{NH}_4^+$ ) generally formed equal fractions, but their concentrations were low (Table 1). The estuarine waters contained two to three times high values of DIP and DISi compared to that of coastal waters (Figs. 3c, d, e & f). The N:P ratio (>10) did not show much variation between the two areas. At the same time, an increase in DISi has led to a substantial increase in the S:N and S:P ratios in the estuary (Table 1).

### *Chlorophyll a*

Chlorophyll *a* (chl *a*) contained in the pico- (0.2 - 2 $\mu\text{m}$ ), nano- (2 - 20 $\mu\text{m}$ ) and micro- (>20 $\mu\text{m}$ ) fractions showed significant variations in the estuarine and coastal waters (Fig. 4). The total chl *a* was higher in the estuary, both in the surface (av.  $13.7 \pm 8 \text{ mgm}^{-3}$ ) and bottom (av.  $9.6 \pm 4.1 \text{ mgm}^{-3}$ ) as compared to the coastal waters (av.  $5.3 \pm 1.8 \text{ mgm}^{-3}$  & av.  $5.9 \pm 3.4 \text{ mgm}^{-3}$ , respectively). The nanoplankton chl *a* was dominated both in the estuary (av. 85.1%) and coastal waters (av. 73.2 %), although its concentration was two times higher in the estuary (surface av.  $10.8 \pm 6.1 \text{ mgm}^{-3}$  & bottom av.  $8.7 \pm 3.9 \text{ mgm}^{-3}$ ) than the coastal waters (surface av.  $3.5 \pm 1.9 \text{ mgm}^{-3}$  & bottom av.  $4.9 \pm 3.1 \text{ mgm}^{-3}$ ). The mean micro- and pico- fractions contributed 8.5 % and 6.5 % of the total chl *a* in the estuary, whereas in the coastal waters it was 15.6 % and 11.2 %, respectively. Similar to nano- fraction, the micro- and pico- chl *a* were also somewhat higher in the estuary (av.  $1.1 \pm 1.08 \text{ mgm}^{-3}$  & av.  $0.77 \pm 0.8 \text{ mgm}^{-3}$ ) as compared to the coastal waters (av.  $0.85 \pm 0.62 \text{ mgm}^{-3}$  & av.  $0.54 \pm 0.4 \text{ mgm}^{-3}$ ).

### *Primary production*

Similar to the distribution of chl *a*, primary production (total and size fractions) also exhibited considerable variation in the estuarine and coastal waters, with higher values in the former (Fig 4). The total primary production showed two fold increase in the estuary (av.  $432 \pm 434 \text{ mgC m}^{-3}\text{d}^{-1}$ ) compared to the coastal waters (av.  $224 \pm 110 \text{ mgC m}^{-3}\text{d}^{-1}$ ) during the study. The nanoplankton primary production was the dominant fraction (av.  $81.2 \pm 3.2 \%$ ) of the total primary production in the estuarine surface (av.  $416.4 \pm 444.5 \text{ mgC m}^{-3}\text{d}^{-1}$ ) and bottom (av.  $290.6 \pm 306.3 \text{ mgC m}^{-3}\text{d}^{-1}$ ) waters. In the coastal waters, the nano-fraction was also dominant (av.  $81.9 \pm 15.7 \%$ ), in the surface (av.  $206.8 \pm 62.5 \text{ mgC m}^{-3}\text{d}^{-1}$ ) and bottom (av.  $173.4 \pm 135.4 \text{ mgC m}^{-3}\text{d}^{-1}$ ) waters, respectively. The microplankton was the second dominant fraction of the total primary production in the estuary (av.  $15 \pm 5.2\%$ ) and coastal

waters (av.  $13.6 \pm 13.7\%$ ) and the production rate was twice in the estuary as compared to the coastal waters. The primary production of the pico-fraction was lower in the estuary and coastal waters as compared to the other fractions (Fig 4). In the estuary, the pico- production rates varied between 1.1 and 49  $\text{mgC m}^{-3}\text{d}^{-1}$  ( $3.8 \pm 2.7 \%$ ) in the surface and bottom waters, whereas in the coastal waters, it varied from 0.6 to 30.7  $\text{mgC m}^{-3}\text{d}^{-1}$  ( $4.6 \pm 2.7 \%$ ).

## Discussion

The spatial variation in the size fractionated phytoplankton biomass and production in the estuarine and coastal waters was distinct in accordance to the environmental changes in the respective regions. In comparison to the coastal waters, the estuary was characterized by freshwater influx, reducing the salinity from 3.1psu to 0.6 psu (during September). The inorganic nutrients such as ammonia (av.  $>6 \mu\text{M}$ ), nitrate (av.  $>12 \mu\text{M}$ ), phosphate (av.  $>3 \mu\text{M}$ ) and silicate (av.  $>70 \mu\text{M}$ ) were consistently high (Fig. 4) during these months with varying concentrations (Table 1). The coastal waters were characterized by as having a high salinity (av.  $>30$  psu) and low nutrient levels. The nutrient enrichment is a common feature of Cochin estuary during monsoon months as the terrestrial run off is supplemented by the fertilizer industry effluents through the Periyar river (Madhu et al, 2007). Alternately, a proportionate decrease in the concentration of DIN, DIP and DISi in the coastal waters maintain the N:P and S:N ratios as similar to that in the estuary (Table 1). However, the high fresh water flow into the estuary increased the S:P (av.  $>20$ ) and S:N (av. 3.5) ratios well above the Red-field ratio in contrast to ratios in the coastal waters (av.  $<15$  & av.  $<2$ ).

The high chl *a* and primary production contributed by the nanoplankton in the estuary (av.  $85 \pm 8.3\%$  & av.  $81.2 \pm 3.2\%$ ) and coastal waters (av.  $73.2 \pm 17\%$  & av.  $81.9 \pm 15.7\%$ ) is evidence of dominance of this community during monsoon season as compared to the autotrophic pico- and microplankton as reported early (Qasim, 1974). Normally nutrient enrichment favours the proliferation of large-sized phytoplankton in the coastal waters ( $>20 \mu\text{m}$ ; Chisholm 1992; Agawin et al., 2000) and also they need high light intensity for saturating photosynthesis (Glover et al., 1985; Cermeno et al., 2005). This was is not seen in the Cochin estuary, as during the monsoon months the daily total radiation in the region is less than  $350 \text{ ly d}^{-1}$  due to the formation of heavy cloud cover and torrential rainfall (av. 400mm per day; Qasim, 2003). In addition, during this season, the increase in the concentration of suspended particulate matter (SPM) due to the terrestrial and riverine run off also lead the reduction of sufficient light for photosynthesis by enhancing water column turbidity (Madhu et al,

2007). According to the earlier report (Madhu et al, 2007), the light attenuation coefficient in the Cochin estuary is higher (av.  $2.4 \pm 0.4 \text{ m}^{-1}$ ) during monsoon compared to the pre-monsoon (av.  $1.2 \pm 0.4 \text{ m}^{-1}$ ). Hence, it is evident that light limitation significantly reduces the photosynthesis of larger phytoplankton in the Cochin estuary during monsoon. Eventhough, microplankton contributed chl *a* is less (av.  $8.5 \pm 4.8\%$ ) in the estuary, their photosynthetic efficiency was relatively high (av.  $7.4 \pm 7 \text{ mgC mg Chl } a \text{ m}^{-3} \text{ hr}^{-1}$ ) as compared to the pico- (av.  $3.1 \pm 4.3 \text{ mgC mg Chl } a \text{ m}^{-3} \text{ hr}^{-1}$ ) and nano- (av.  $2.9 \pm 1.8 \text{ mgC mg Chl } a \text{ m}^{-3} \text{ hr}^{-1}$ ) fractions, due to their large size and phyletic affiliation (Taylor et al, 1997). However, in the coastal waters, nanofraction showed maximum photosynthetic efficiency (av.  $4.8 \pm 3.9 \text{ mgC mg Chl } a \text{ m}^{-3} \text{ hr}^{-1}$ ) than micro- (av.  $3.7 \pm 3.5 \text{ mgC mg Chl } a \text{ m}^{-3} \text{ hr}^{-1}$ ) and pico- (av.  $2.5 \pm 4.2 \text{ mgC mg Chl } a \text{ m}^{-3} \text{ hr}^{-1}$ ) fractions, eventhough the percentage of the microplankton chl *a* (av.  $15.6 \pm 12.4 \%$ ) seems to be slightly higher than the estuary.

It is known that nanoplankton are more efficient at using low light intensities and ambient nutrient concentrations for photosynthesis than larger phytoplankton (Shiomoto, 1997). The present study shows that nanoplankton contributed greatly to chl *a* and primary production of the estuary (av. 85% & av. 81%) and coastal waters (av. 73% & av. 82%) during the monsoon as compared to other fractions (pico- and micro-). The coincidence of low nanoplankton chl *a* and primary production in the coastal waters (compared to estuary) is due to the active grazing of protozoans (ciliates and flagellates) thriving in the moderately saline waters ( $>30 \text{ psu}$ ; Riegman et al., 1993; Jyothibabu et al, 2006). It can be concluded that the heavy cloud cover along with torrential rainfall and increased water column turbidity significantly prevents solar radiation in the study area during monsoon and thus limits the growth of large sized phytoplankton.

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Table 1- Variability (standard deviation) of DIN and the nutrient ratios in the estuary and coastal waters during the monsoon months, 2006

	Estuary		Coastal	
	Surface	Bottom	Surface	Bottom
NO <sub>2</sub> <sup>-</sup> (μM)	0.3 ± 0.2	0.6 ± 0.3	0.3 ± 0.3	0.4 ± 0.2
NO <sub>3</sub> <sup>-</sup> (μM)	12.5 ± 4.5	12.4 ± 2.9	1.6 ± 0.5	2.9 ± 3.0
NH <sub>4</sub> <sup>+</sup> (μM)	6.4 ± 3.1	7.5 ± 3.3	2.1 ± 1.0	1.0 ± 0.2
N:P	6.2 ± 2.2	7.5 ± 1.3	9.7 ± 9.6	4.4 ± 3.8
S:N	4.1 ± 2.4	3.7 ± 2.5	2.2 ± 2.0	1.5 ± 1.3
S:P	24.9 ± 14.5	29.5 ± 24	14.6 ± 9.2	5.7 ± 3.1

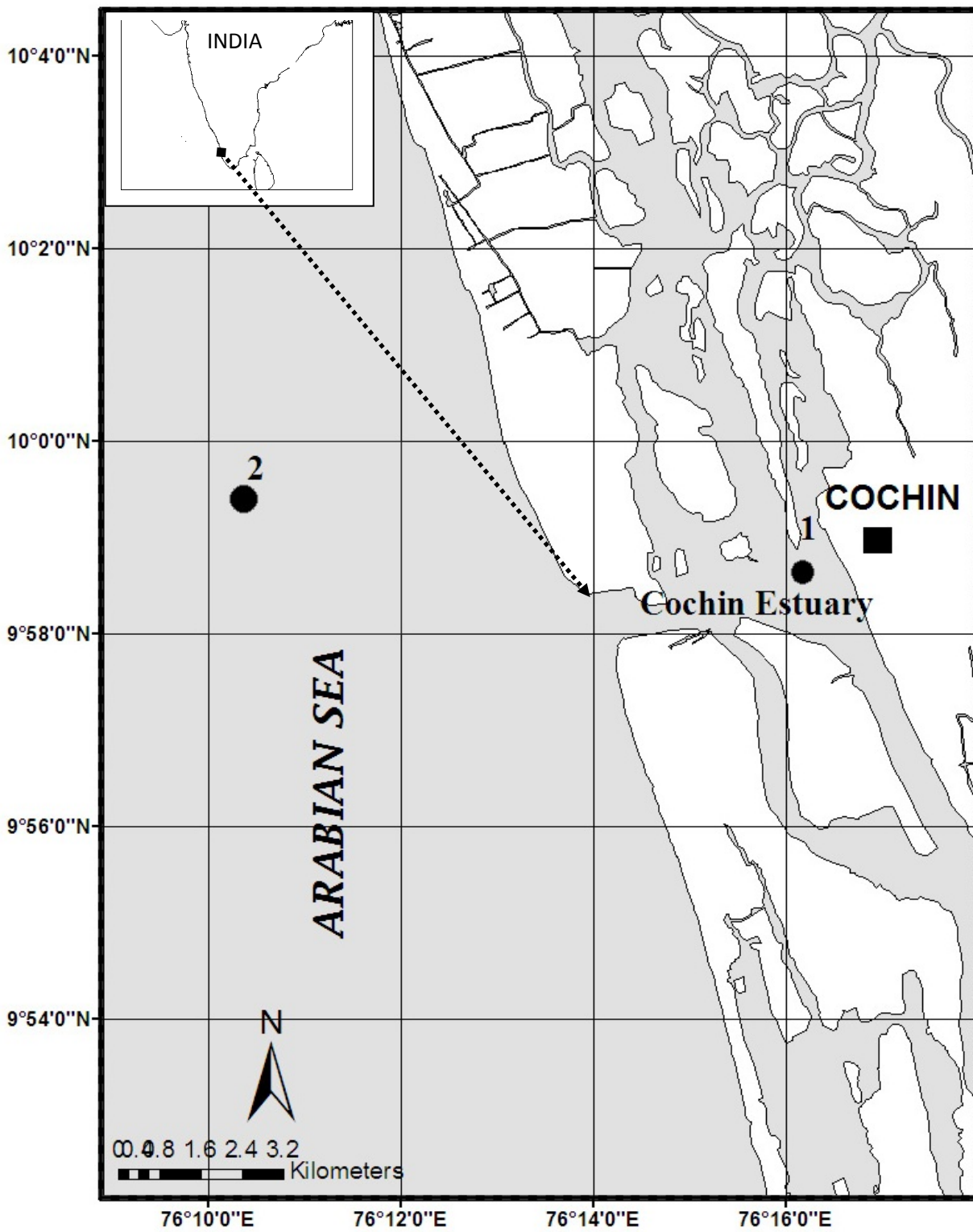


Figure 1 - Station locations

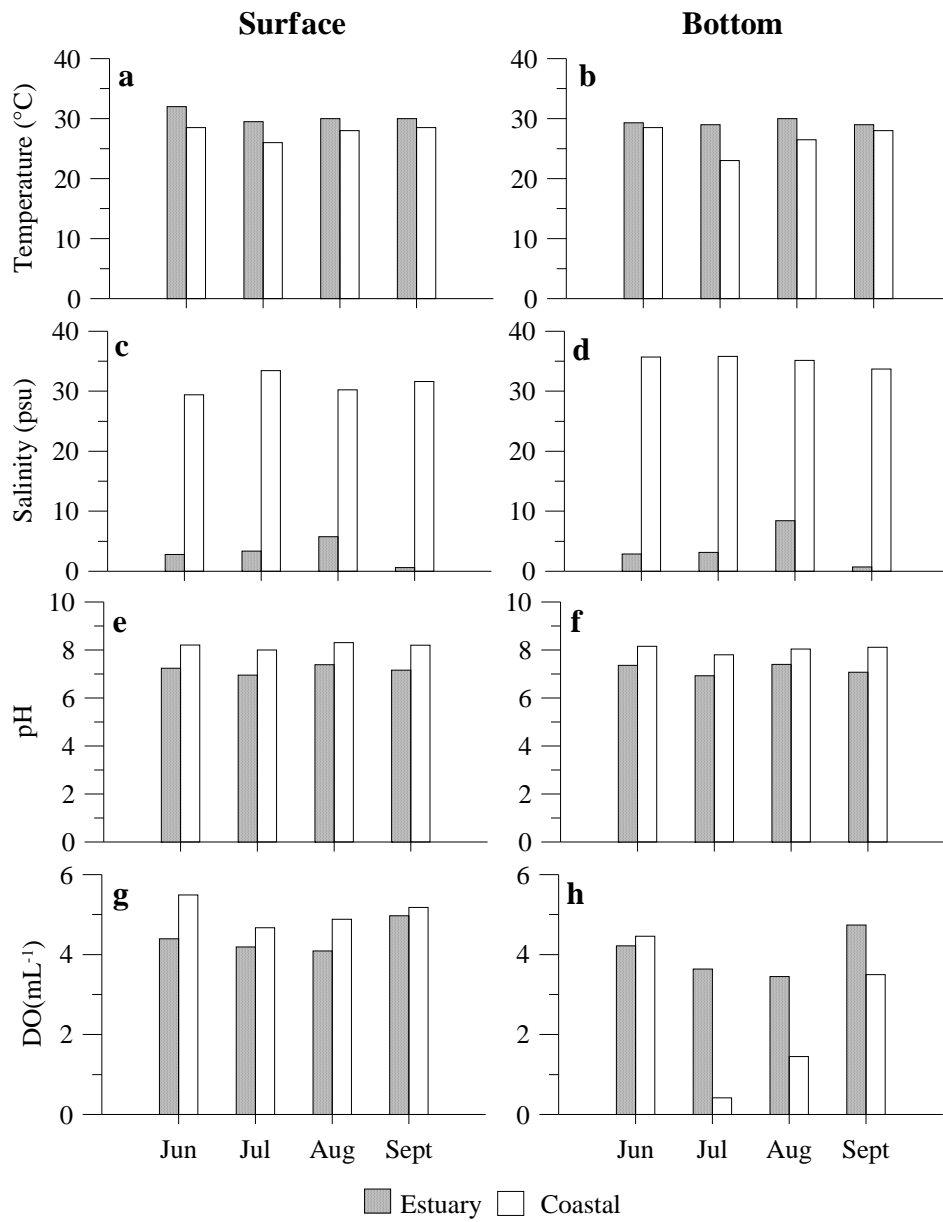


Figure 2 - Distribution of temperature, salinity, pH and DO in the Cochin estuary and coastal waters during the monsoon months

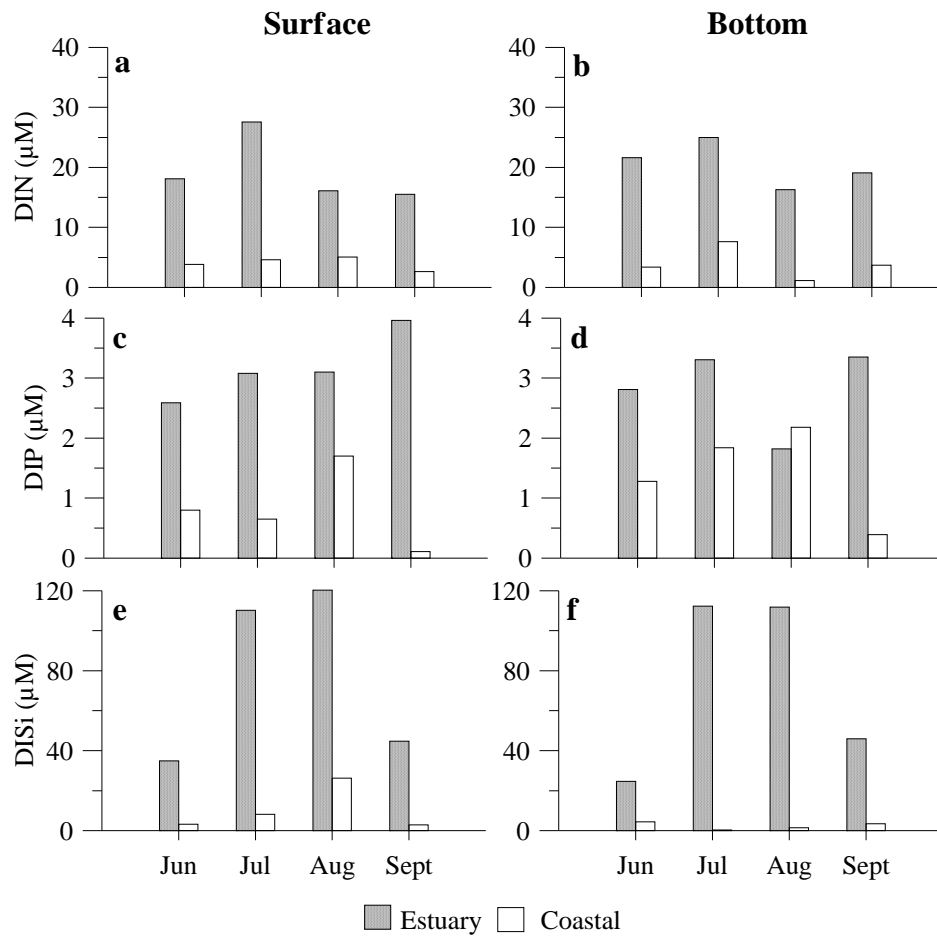


Figure 3 - Distribution of DIN, DIP, DISi in the Cochin estuary and coastal waters during the monsoon months

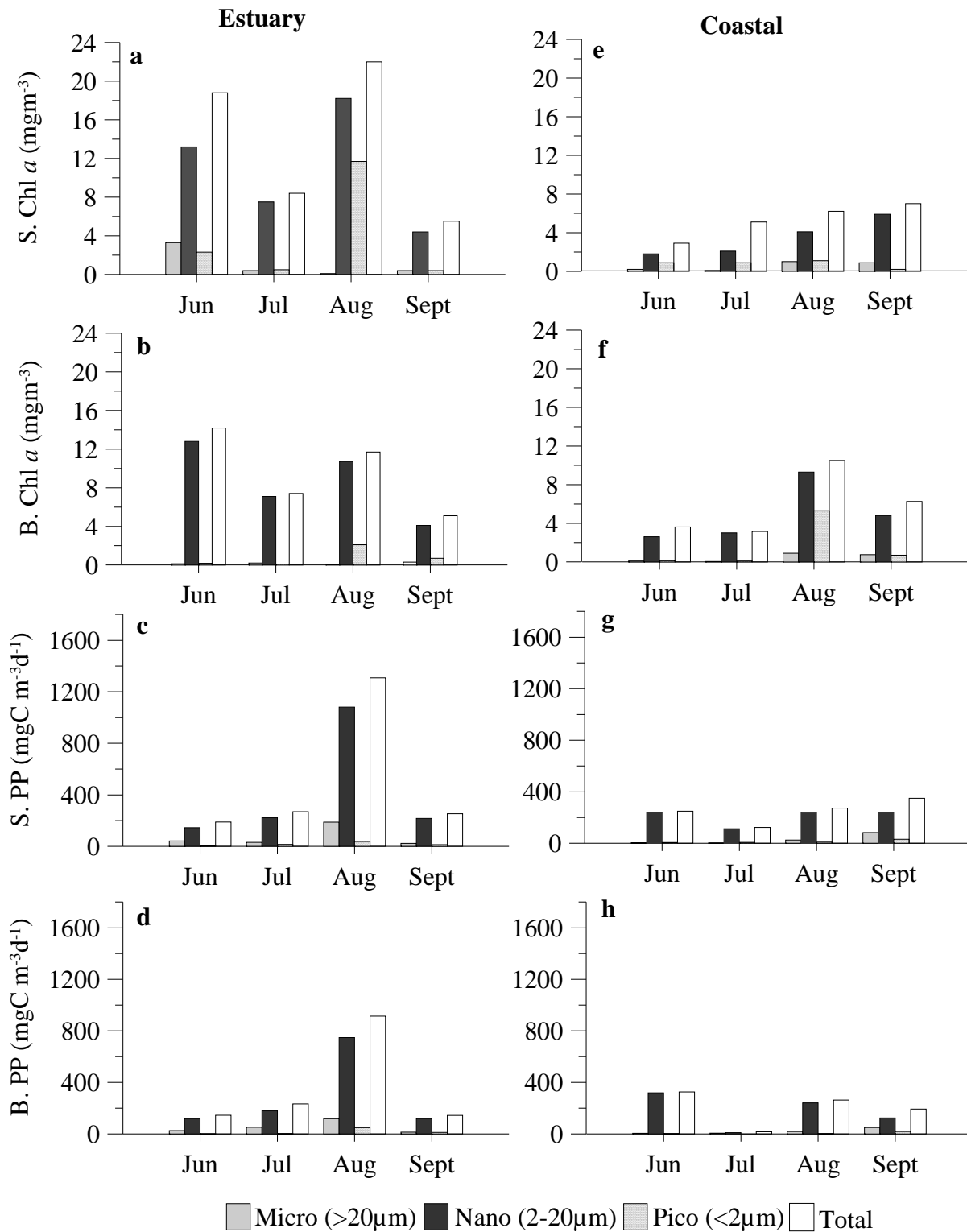


Figure 4 - Distribution of size fractionated chlorophyll *a* and primary production in the Cochin estuary and coastal waters during the monsoon months