

Summer Monsoon Onset -induced Changes of Autotrophic Pico - and Nano - plankton in the Largest Monsoonal Estuary along the West Coast of India

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Abstract

This study presents the response of autotrophic pico- and nanoplankton to Southwest Monsoon-associated hydrographical transformations in the Cochin backwaters (CBW), the largest monsoonal estuary along the west coast of India. By the onset of the Southwest Monsoon, the euhaline/mesohaline conditions in the downstream/upstream of CBW usually transform into oligohaline/limnohaline. The flow cytometer analysis revealed the dominance of Picoeukaryotes > *Synechococcus* > Nanoautotrophs, with *Prochlorococcus* either very low or entirely absent. *Synechococcus* abundance was high during the Pre-Southwest Monsoon (10^6 L^{-1}), which dwindled with heavy fresh water influx during the Southwest Monsoon (10^5 L^{-1}). The drastic drop in salinity and faster flushing of the CBW during the Southwest Monsoon replaced the euhaline/mesohaline strain of *Synechococcus* with an oligohaline/limnohaline strain. Epifluorescence microscopy analyses showed that, among the two strains of *Synechococcus*, the phycoerythrin-rich one was dominant in the mesohaline/ euhaline conditions, whereas the phycocyanin-rich strain dominated in oligohaline / limnohaline conditions. Although *Synechococcus* abundance diminished during the Southwest Monsoon, the total abundance of picoplankton community remained virtually unchanged in the upstream due to an increase in the abundance of Picoeukaryotes. On the other hand, the autotrophic nanoplankton abundance increased from Pre-Monsoon levels of av. $3.8 \times 10^6 \text{ L}^{-1}$ to av. $9.5 \times 10^6 \text{ L}^{-1}$ at the onset of the Southwest Monsoon. Utilizing suitable multivariate analyses, the study illustrated the differential response and niche preference of various smaller communities of autotrophs to the Southwest Monsoon-associated hydrographical ramifications in a large monsoonal estuary, which may be applicable to similar such estuaries situated along the Indian coastline.

Keywords: Phytoplankton, *Synechococcus*, *Prochlorococcus*, Picoeukaryotes, Cochin Backwaters

1. Introduction

Smaller autotrophic plankton consist of pico - (0.2 - 2 μm) and nano - (2 - 20 μm) size ranges inhabiting the euphotic ocean surface layer. Earlier studies showed that autotrophic picoplankton occurs abundantly in the oceanic/oligotrophic environments where they contribute more than 50% of the gross primary production (Stockner and Antia 1986; Li 1983; Li and Wood 1988). More recent research evidenced that autotrophic picoplankton are abundant in the nutrient-rich waters such as coastal and estuarine waters as well, though their proportionate contribution to the gross primary production in such environments is lower than the large-sized phytoplankton (Philip et al. 1999; Badylak and Philips 2004; Mitbavkar and Anil 2011; Mitbavkar et al. 2012). On the other hand, the significant contribution of nanoplankton to the gross phytoplankton biomass and production in nutrient-rich coastal and estuarine waters is well accepted (Qasim 2003; Madhu et al. 2007; Jyothibabu et al. 2013; 2014). In recent years, the presence and ecological role of the smaller phytoplankton in marine ecosystem are increasingly accepted, as they form the base of the complex microbial plankton food web, the important functional entity in recycling and transfer of organic matter to the higher trophic levels (Pomeroy 1974; Azam et al. 1983; Lampitt et al. 1993; Cermeno et al., 2006).

Autotrophic picoplankton generally consists of *Prochlorococcus*, *Synechococcus* and picoeukaryotes. *Prochlorococcus* and *Synechococcus* are considered the most abundant photosynthetic organisms in the ocean, contributing significantly to the global primary production (Veldhuis et al. 1993; Burkill et al. 1993; Partensky et al. 1996). Further studies on this subject identified several strains of *Synechococcus* from rivers, lakes, estuaries and seas from various parts of the world, leading to the general belief that *Synechococcus* are more diverse than *Prochlorococcus* (Fuller et al. 2003; Ahlgren and Rocop 2006; Mitbavkar et al. 2012). Available information on the taxonomic composition and diversity of autotrophic pico-eukaryotes and nanoplankton is considerably lesser than autotrophic picoplankton. Available information indicates autotrophic flagellates as the main component of pico-eukaryotes, whereas smaller diatoms also contribute to the autotrophic nanoplankton community (Fuller et al. 2003; Ahlgren and Rocop 2006; Mitbavkar et al. 2012).

The present study area, Cochin backwaters/estuary, is significantly influenced by heavy rainfall during the Southwest / Summer Monsoon (June - September) and, therefore, categorized as a typical Monsoonal Estuary where the total water inflow into the system is several orders of magnitude larger than the estuarine volume (Vijith et al. 2009). Information regarding the ecology and distribution of autotrophic pico- and nanoplankton and their response to the Southwest Monsoon rainfall and

freshwater influx in the Cochin backwaters remains vague and incomplete considering that relevant studies addressing the issue have not been undertaken. As large hydrographical changes are usual in the Cochin backwaters during the Southwest Monsoon, attempts are made in this paper to understand the response of the smaller autotrophic plankton to rapid hydrographical changes. Using state-of-the-art equipment such as Flow Cytometry and Fluorescence Microscopy, we studied the autotrophic picoplankton and nanoplankton in the Cochin backwaters to understand their (a) distribution ecology in different salinities from marine to freshwater conditions during the Pre-Southwest Monsoon (b) their response to significant hydrographical changes that occur during the onset of the Southwest Monsoon and (c) their distribution pattern during limnohaline/limnetic conditions that ensue throughout the Southwest Monsoon.

2. Materials and Methods

2.1 Study Area

Cochin backwaters/estuary (CBW) is the largest of its kind along the west coast of India (Figure 1). It is situated between 9°30' to 10°20'N and 76°13' to 76°5'E. The estuary occupies an area of 130 km² and has two permanent inlets into the Arabian Sea – a wider one at Kochi compared to the other at Azhikode in the north. Tidal incursion from the Arabian Sea (tidal range of ~1m) contributes a regular flow of salt water into the Cochin backwaters. Tidal influence is dominant during the non-monsoon period and more emphatic in the downstream region; its amplitude diminishes considerably towards the upstream (Madhupratap 1987). The main source of freshwater into the Cochin backwaters are six rivers (Pamba, Achancoil, Manimala, Meenachil, Periyar and Muvattupuzha), of which the first three drain into the southern part of the system.

Typically, the Southwest Monsoon rainfall begins in the study domain during the first week of June and peaks during mid-June to August (Qasim 2003). During the peak Southwest Monsoon, heavy rainfall (40 - 50 cm in a few hours) occurs in the region (Qasim 2003) and salinity reaches near zero values over most parts of the CBW (Jyothibabu et al. 2006). The gross phytoplankton biomass and production in the Cochin backwaters are very high and remain largely constant all through the year, due to a qualitative shift in the phytoplankton community during the Southwest Monsoon conditions (Qasim 2003; Jyothibabu et al. 2006). In order to develop conclusive information on the response of smaller plankton to various hydrographical settings in the Cochin backwaters, we adopted the standard salinity grouping designed for estuarine conditions by McLusky (1993), based on which waters/zones were grouped as (a) euhaline (salinity > 30), (b) polyhaline (salinity 25 to 32), (c) mesohaline (salinity 5 to 18), (d) oligohaline (salinity 0.5 to 5) and (e) limnetic (salinity < 0.5).

2.2. Sampling

Altogether 14 locations were sampled along the salinity gradients in the Cochin backwaters. The frequency of sampling in the study area was designed in such a way that the community response of smaller autotrophic plankton to stratified/high saline conditions during the Pre-Southwest Monsoon, hydrographical transition phase during the onset of the Southwest Monsoon and the limnohaline/limnetic conditions during the Southwest Monsoon could be quantified. In order to accomplish this, a typical Pre-Southwest Monsoon hydrographical setting in the Cochin backwaters was sampled on May 26, 2011. The second sampling in the study area was carried out on June 3, 2011, intended to capture the signals of hydrographical transition from the Pre-Southwest Monsoon to the Southwest Monsoon. Typical limnohaline conditions existing in the Cochin backwaters during the Southwest Monsoon were captured on June 9 and 15 in 2011. The field sampling was carried out on a power boat, and the sampling began at Kochi (location 1) by 10AM and ended at Thannermukkom (location 14) by 1 PM (Figure 1). A SEACAT SBE 19 plus V2 portable CTD (Conductivity, Temperature, and Depth) profiler was used to record the vertical salinity and DO (dissolved oxygen) distributions in locations. As the study area is shallow, water samples were collected from just below the surface (~1m) using a Niskin sampler and used for quantifying various chemical and biological parameters.

2.3. Climatic and Environmental Factors

Rainfall data representing the study domain was obtained from Indian Meteorological Department, Pune. The daily rainfall data for the two major revenue districts encompassing the Cochin backwaters (Ernakulum and Alappuzha) were considered in the present study to show the drastic increase in rainfall and its connection to the hydrographical transformations by the onset of the Southwest Monsoon. The salinity values recorded by CTD sensors was presented to show the rapid hydrographical changes that occur in the Cochin backwaters as the Southwest Monsoon sets in by the first week of June. The turbidity levels in different sampling locations were measured using a turbidity meter (Eutech Model TN 100) based on nephelometric principles. Dissolved inorganic macronutrients (Nitrate – NO_3 , Phosphate - PO_4 , and Silicate - SiO_4) were measured following standard colorimetric techniques (Grasshoff et al. 1983).

2.4. Biological Parameters

Water samples for the analysis of phytoplankton total chlorophyll a was filtered through Millipore membrane filters (0.2 μm), followed by chlorophyll extraction with 90% acetone and its measurement by a Trilogy Turner fluorometer following standard procedure (UNESCO 1994). Flow

Cytometric samples of autotrophic pico – and nanoplankton populations were preserved with 1% glutaraldehyde solution and stored in liquid nitrogen until analysis was initiated (Vaulot et al. 1996). Prior to the analysis, batches of thawed samples were homogenized with a laboratory test tube shaker. Samples were then analyzed in a BD Accuri C6 Flow Cytometer to quantify autotrophic pico - and nanoplankton. Yellow-green fluorescent beads (2 μm and 6 μm , Polysciences) were used to standardize the measurements. The Flow Cytometry facilitated the quantification of autotrophic smaller plankton based on the forward light scatter (FLS; proxy for cell size) and cellular fluorescence from photosynthetic pigments (chlorophyll *a*, phycoerythrin and phycocyanin). For confirmation of the Flow Cytometric measurements, microscope slides were prepared randomly based on standard procedures and scanned under an Epifluorescence microscope (Olympus IX 71) with a blue excitation filter set (Bloem et al. 1986). The present study considered yellow-orange fluorescence emission for the phycoerythrin-rich *Synechococcus* strain and red for the the phycocyanin-rich one, as observed under a blue excitation light (Ernst 1991; Callieri et al. 1996).

2.5. Statistical Analyses

2.5.1. Cluster / SIMPROF and NMDS

Cluster/ SIMPER and NMDS were used to segregate the observations of different parameters into clusters based on their similarity/homogeneity. The data or observations in one cluster indicate their similarity whereas their placement in different clusters show their dissimilarity. The data of plankton components were initially standardized and $\log(X+1)$ transformed to normalize the differences in numerical abundance (Clarke and Warwick 1994).

2.5.2. Redundancy Analysis (RDA)

RDA was used to elucidate the interrelationships between the smaller autotrophic plankton components and their environmental variables. Initially, the data was processed using Detrended Correspondence Analysis (DCA) to select the suitable ordination technique. The result of DCA showed axis gradient length < 2 , which suggest linear multivariate RDA suitable for the present data (Birks 1998; Leps and Smilauer 2003). The biological variables were log transformed prior to the analysis. The ordination significance was tested with Monte Carlo permutation tests (499 unrestricted permutations) ($p < 0.05$). The results of the RDA are presented in tri-plots in which the samples are displayed by points and environmental and biological variables by arrows. Arrows for environmental and biological variables indicate the direction in which the corresponding variable increases (Leps and Smilauer 2003).

3. Results

3.1. Rainfall and Environmental Parameters

The pattern of rainfall during the study period from May 22 to June 15 in 2011 is presented in Figure 2a. Similarly, the distribution of salinity and dissolved oxygen in the Cochin backwaters recorded during the study period has been depicted in Figure 2b-e. It is evident that during the Pre-Southwest Monsoon the study area was characterized by very low / nil rainfall with stratified water column. The CTD profiles during the period presented typical estuarine features in the study domain with polyhaline conditions in the downstream stretches (location 1 and 2), mesohaline conditions in the middle stream locations (locations 3 to 8) and oligohaline conditions in the upstream region (locations 9 to 14).

The Southwest Monsoon rainfall in the study domain began on 30th May, a few days earlier than expected. Heavy rainfall was recorded during the onset of the Southwest Monsoon rainfall in the first week of June and later it diminished erratically with highs and lows. The rapid increase in rainfall during the onset of the Southwest Monsoon caused a drastic decline in the salinity of the study domain. During Monsoon 1 sampling in the first week of June, the Cochin backwaters transformed into limnohaline/ oligohaline in the upstream/downstream stretches and presented a drastic decline in the average salinity in the study area (salinity av. 0.49 ± 0.9) (Figure 2 and Table 1). Oligohaline conditions prevailed in locations 1 to 6 (salinity 0.66 - 1.24), and limnohaline conditions in locations 7 to 14 (salinity <0.5).

The average salinity in the Cochin backwaters further decreased during Monsoon 2 sampling and was the lowest of all (av. 0.26 ± 1.9), with oligohaline conditions prevailing in locations 1 and 2 and limnetic conditions in the rest (locations 3 to 14). The average salinity in the system Monsoon 2 (salinity av. 0.26 ± 1.9) was found to be even lower during the Monsoon 3 collection (salinity av. 0.28 ± 1.7). During Monsoon 3 collection, oligohalinity prevailed in locations 1 to 3 and limnetic conditions in the others (locations 4 to 14). The turbidity values recorded during different collections are presented in Table 1. The water column during the Pre-Southwest Monsoon was clearest among all the observations, as evidenced by the lowest turbidity values during the study period (Table 1). The turbidity level significantly increased as the Southwest Monsoon progressed in the study domain. The turbidity in the study area was the highest during Monsoon 1 (av. 40.65 ± 0.8 NTU), decreased during Monsoon 2 (av. 14.9 ± 0.6 NTU) and then increased during Monsoon 3 (av. 24.2 ± 1.1 NTU).

3.2. Silicate, Nitrate and Phosphate

Silicate was high in the Cochin backwaters throughout the observations. The average silicate concentration in the study area was high even during the Pre-Southwest monsoon (av. 38.64 ± 9.62 μM), the period with lowest river discharge (Table 1). Silicate concentration sequentially increased from Pre-Southwest Monsoon (av. 38.6 ± 0.2) to Monsoon 1 (av. 111.95 ± 0.1 μM) to Monsoon 2 (av. 117.55 ± 0.1 μM) and Monsoon 3 (av. 128.50 ± 0.1 μM). Like silicate, the nitrate level was also high in the Cochin backwaters throughout the sampling session and a clear sequential increase from one session to the next was not evident. The nitrate concentration was the highest of all sampling during Monsoon 3 (av. 27.78 ± 0.2 μM) followed by Pre-Monsoon (av. 20.31 ± 0.6 μM), Monsoon 2 (av. 16.98 ± 0.1 μM) and Monsoon 1 (av. 9.44 ± 0.5 μM). The concentration of phosphate was found to be low and comparable during all the sampling sessions. The lowest phosphate concentration was found during Monsoon 3 (av. 0.1 ± 0.6 μM), followed by Pre-Southwest Monsoon (av. 0.227 ± 0.5 μM), Monsoon 1 (av. 0.240 ± 0.5 μM) and Monsoon 2 (av. 0.3 ± 0.3 μM).

3.3. Segregation of Locations Based on Environmental Parameters

Based on the environmental parameters recorded in 14 locations in the Cochin backwaters during the weekly sampling, several clusters of locations were identified. During the Pre – Southwest Monsoon sampling, 3 clusters of locations were identified in the study area (Figure 3 a-c). Locations close to the Kochi inlet (locations 1 and 2) were assembled in cluster 1, which was characterized by polyhaline waters with higher concentrations of phosphate, lower concentrations of nitrate and lower turbidity compared to the rest of the locations. Locations in the mesohaline waters with moderate levels of silicate and turbidity were grouped as cluster 2 (locations 3 to 8). Locations in the oligohaline waters with the highest level of silicate and turbidity and the lowest concentration of phosphate were grouped as cluster 3 (locations 9 to 14).

During Monsoon 1 sampling, a significant change was noticeable in the hydrography of the Cochin backwaters from that of the Pre–Southwest Monsoon conditions (Table 1). During this period, locations in the entire study area were grouped into two clusters: locations closer to the Kochi inlet under some influence of saltwater intrusion from the Arabian sea formed cluster 1 (locations 1 to 6) and the remaining locations dominated by freshwater (locations 7 to 14) formed cluster 2 (Figure 3d-f). The cluster 1 locations were oligohaline with relatively high phosphate and low nitrate, silicate and turbidity. Oligohaline waters with high silicate and turbidity and low phosphate prevailed in cluster 2.

During Monsoon 2 sampling, saline water intrusion was confined only to locations very close to the Kochi inlet (Figure 4a-c). As a result, the entire sampling locations were characterized by a small cluster 1 (locations 1 and 2) and a large cluster 2 (locations 3 to 14). Cluster 1 was characterized by oligohaline conditions with low nitrate and turbidity, and high silicate. On the other hand, cluster 2 showed limnetic conditions with high silicate, nitrate and turbidity. The clustering of locations based on Monsoon 3 collection was considerably similar to Monsoon 2 with a small cluster (cluster 1) formed of locations 1 to 3 and a large cluster (cluster 2) formed of locations 4 to 14 (Figure 4d-f). Evidently, cluster 1 represented the oligohaline conditions and cluster 2 limnohaline (Figure 4f).

3.4. Biological factors

3.4.1. Chlorophyll a

The chlorophyll values were generally high in the study area ($>1.5 \text{ mg m}^{-3}$) in all the weekly sampling sessions (Table 1). The temporal variations in chlorophyll during the four weekly sampling sessions showed relatively high values in the Pre-Monsoon period (av. $4.81 \pm 0.6 \text{ mgm}^{-3}$), a peak in Monsoon 1 (av. $12.56 \pm 0.6 \text{ mgm}^{-3}$), and a subsequent drop in Monsoon 2 (av. $2.68 \pm 0.3 \text{ mgm}^{-3}$) and Monsoon 3 (av. $1.65 \pm 0.8 \text{ mgm}^{-3}$). Like chlorophyll, phaeopigments were also high in the study area throughout the observations.

3.4.2. *Synechococcus*

A typical flowcytogram of pico - and nanoplankton community inhabiting the Cochin backwaters is presented in Figure 6a. The samples collected during the four weekly sampling sessions showed the highest abundance of *Synechococcus* during the Pre-Monsoon sampling (av. $41.5 \pm 0.8 \times 10^5 \text{ L}^{-1}$) (Figure 5). In the subsequent sampling sessions, *Synechococcus* cell abundance markedly diminished, as evident during Monsoon 1 (av. $9.7 \pm 0.6 \times 10^5 \text{ L}^{-1}$), Monsoon 2 (av. $8.5 \pm 0.5 \times 10^5 \text{ L}^{-1}$) and Monsoon 3 (av. $2.2 \pm 0.4 \times 10^5 \text{ L}^{-1}$).

The random analysis of the picoplankton samples using epifluorescence microscopy showed a qualitative shift in *Synechococcus* community in different salinity regimes (Figure 7). The phycoerythrin-dominant community (yellow-orange color) was dominant during the pre Southwest Monsoon (high saline) conditions, whereas it was a phycocyanin-dominant one (red color) during the Southwest Monsoon (low saline/ limnetic) conditions. Even though a qualitative shift in *Synechococcus* community occurred in the Cochin backwaters during the Southwest Monsoon, their total abundance was strongly linked to the salinity distributions. High saline conditions favored the abundance of *Synechococcus* community whereas Southwest Monsoon conditions declined their total abundance (Figure 8).

3.4.3. Picoeukaryotes

The picoeukaryotes community represented in the flow cytogram is depicted in Figure 6. The pattern of distribution of the pico-eukaryotes was different from that of the *Synechococcus* (Figure 8, Table 1). The abundance of pico-eukaryotes was higher during the Pre-Monsoon (av. $2.1 \pm 0.9 \times 10^7 \text{ L}^{-1}$) as compared to Monsoon 1 (av. $1.6 \pm 0.5 \times 10^7 \text{ L}^{-1}$ cells ml^{-1}). However, during the subsequent sampling in Southwest Monsoon, the pico-eukaryotes abundance increased, as found during Monsoon 2 (av. $3.2 \pm 0.9 \times 10^7 \text{ L}^{-1}$) and Monsoon 3 (av. $3.8 \pm 1.2 \times 10^7 \text{ L}^{-1}$). Though there was a decline in the abundance of pico-eukaryotes during the period of Monsoon 1 sampling, their overall abundance increased as a response to high freshwater influx (Figure 8).

3.4.4. Autotrophic Nanoplankton

Autotrophic nanoplankton temporal distribution pattern was similar to autotrophic pico-eukaryotes. The abundance of nanoplankton was high during the Pre-Southwest Monsoon (av. $3.8 \pm 0.8 \times 10^6 \text{ L}^{-1}$) as compared to Monsoon 1 (av. $3.1 \pm 1.2 \times 10^6 \text{ L}^{-1}$). However, the abundance of autotrophic nanoplankton significantly increased in the subsequent sampling sessions during Monsoon 2 (av. $10.6 \pm 0.5 \times 10^6 \text{ L}^{-1}$) and Monsoon 3 (av. $9.7 \pm 1.5 \times 10^6 \text{ L}^{-1}$). Similar to the autotrophic pico-eukaryotes, the abundance of autotrophic nanoplankton displayed a negative relationship with salinity.

3.4.5. *Prochlorococcus*

The abundance of *Prochlorococcus* was either negligible or absent in the Cochin backwaters. Very low occurrence of the organism was noticed in locations in close proximity with the inlets, and that too without any clear temporal trend. As their occurrence was highly irregular and confined to a few locations in the inlet locations, their distribution and interrelationship with salinity was not attempted by statistical treatments involved in Tri-plot (Figure 9).

4. Discussion

4.1. Hydrographical Transformation during the Southwest Monsoon

It is well accepted that the downstream of the Cochin backwaters behaves as an extension of the Arabian Sea during the Pre-Southwest Monsoon due to active intrusion of saline water while the upstream remains in low saline conditions due to fresh water influx from rivers (Madhupratap 1987). This typical estuarine hydrographical setting in the Cochin backwaters was prevalent during the present Pre-Southwest Monsoon sampling also. During this period, the hydrography of the Cochin backwaters showed polyhaline waters in the downstream stretches, mesohaline waters in the central

part and oligohaline waters in the upstream regions, as evident in the cluster analysis of locations based on similarities in their environmental factors. This essentially represents a gradual dilution of high saline Southeastern Arabian Sea waters (~34) from the downstream to the upstream of the Cochin backwaters during the Pre-Southwest Monsoon.

The southwest Monsoon rainfall in the study domain typically begins during the first week of June, which rapidly transforms the Cochin backwaters from truly estuarine to limnetic (Qasim 2003; Jyothibabu et al. 2004). This feature was clearly captured in the present sampling; as a few days of heavy rainfall during the first week of June significantly transformed the hydrography of the Cochin backwaters into a fresh water dominant system with oligohaline conditions in the downstream and limnetic conditions in the upstream during Monsoon 1 sampling. The dominance of freshwater in the Cochin backwaters further increased during Monsoon 2 and 3 sampling sessions, causing a few downstream locations to become oligohaline and turning all the rest of the locations limnohaline. This indicates that the onset of the Southwest Monsoon rainfall causes rapid transformation of the study domain into a freshwater dominant one. As monsoon rainfall persists for longer time scales, the entire Cochin backwater area becomes dominated by the freshwater with only a few downstream locations remaining oligohaline and the rest turning limnohaline.

4.2. Response of plankton to hydrographical changes

Significantly high levels of nitrate and silicate, primarily contributed by several rivers that empty into the system, are usual in the Cochin backwaters all through the year, (Jyothibabu et al. 2006). There are several non-point sources such as agricultural and aquaculture along the banks of the Cochin backwaters that too contribute to the nutrient budget of the system (Saraladevi et al. 1983). The high nutrient level in the Cochin backwaters supports significantly high phytoplankton biomass, mainly contributed by nanoplankton (Madhu et al. 2007; Jyothibabu et al. 2014). This typical feature in nitrate and silicate levels of the Cochin backwaters was reflected well in the present study also as there were significantly high levels of nitrate and silicate throughout the sampling session.

During the Pre-Monsoon sampling, even with surplus nutrients available in the Cochin backwaters, the total phytoplankton biomass was moderate (av. 4.8 mg m⁻³) compared to the Monsoon 1 sampling session (av. 12.6 mg m⁻³). The relatively stable environmental conditions during the Pre-Monsoon period allows the zooplankton to diversify and increase their stock, causing a more effective feeding interaction and grazing pressure on phytoplankton (Madhupratap et al. 1987; Jyothibabu et al. 2014). The increased zooplankton grazing of phytoplankton (top down control) could be a potential reason for the moderate level of chlorophyll *a* during the Pre-Monsoon sampling

when environmental conditions remained conducive for phytoplankton growth. Earlier studies have showed that herbivorous copepods and microzooplankton consume a major part of the phytoplankton stock during high saline and relatively stable hydrographical settings (Tan et al. 2004; Jyothibabu et al., 2006). On the other hand, much of the phytoplankton stock is left unconsumed in the Cochin backwaters during the Southwest Monsoon conditions due to significantly low occurrence of microzooplankton (1/9 of the Pre-Monsoon) and herbivorous meso-zooplankton (1/8 times of the Pre-Monsoon).

The qualitative shift in larger phytoplankton community in the Cochin backwaters associated with the Southwest Monsoon has been noticed in many studies (Qasim et al. 2003; Jyothibabu et al. 2006). During the onset of the Southwest Monsoon, a large phytoplankton community dominated by chain-forming diatoms from the upstream freshwater region are transported downstream and become widespread in the Cochin backwaters. This feature was consistent with the present observation in which a remarkable increase in total chlorophyll was observed during the Monsoon 1 sampling. The snapshots of the microscope slides showing large chain-forming phytoplankton community during Monsoon 1 sampling is presented as supplementary material 1. This provides clear evidence for the predominance of large chain-forming diatoms during the onset of the Southwest Monsoon, contributing significantly to a significant increase in the total chlorophyll concentration ($>10\text{mgm}^{-3}$) during the period.

4.2.1. Response of autotrophic pico- and nanoplankton

In tropical estuarine waters, the contribution of *Synechococcus* to the total phytoplankton carbon biomass ranges from 9 to 29% and considered to be an indication of the estuarine hydrodynamics (Mitbavkar and Anil 2011; Mitbavkar et al. 2013). During the Pre-Southwest Monsoon, the abundance of *Synechococcus* was significantly higher in the polyhaline/limnetic conditions in the Cochin backwaters. In marine environments, *Synechococcus* prefers nutrient-rich environments and occurs abundantly in the well-lit portion of the euphotic zone (Partensky 1996). During the Southwest Monsoon, a qualitative shift in *Synechococcus* community was evident in the Cochin backwaters with phycoerythrin-rich (PE-rich) marine strains abundant in the polyhaline downstream region and phycocyanin-rich (PC-rich) mesohaline/limnohaline strain in the low saline/upstream regions. Similarly, the highest abundance of *Synechococcus* was found in the mesohaline region (location 3-7) of the Cochin backwaters during the Pre-Southwest Monsoon, where both strains contributed to the high abundance. It was observed earlier that *Synechococcus* strain abundant in oceanic waters are phycoerythrin-rich (PE-rich) while those in turbid fresh waters are phycocyanin-rich (PC-rich) (Wood et al. 1985; Pick 1991; Voros et al. 1998; Stomp et al. 2007; Wang et al. 2011).

Studies showed that several ecotypes/strains of prokaryotic picoplankton increase the adaptability of a species in different hydrographical settings (Partensky et al. 1999; Fuller et al. 2003).

Intense rainfall during the onset of the Southwest Monsoon rapidly transforms the hydrography of the Cochin backwaters from polyhaline/mesohaline conditions to limnetic conditions in the downstream/upstream regions (Madhupratap 1987; Qasim 2003). The significant drop in salinity by large freshwater influx led to a change in the community structure of the smaller autotrophic plankton as well. As the Southwest Monsoon rainfall sets in the study domain, the abundance of *Synechococcus* significantly declined in the entire study area during all monsoon collections. On the other hand, the abundance of autotrophic pico-eukaryotes and nanoplankton declined during Monsoon 1, but subsequently increased during the Monsoon 2 and Monsoon 3 sessions. This indicates a possible shift in the qualitative composition of both components during the Southwest Monsoon conditions. However, this aspect is beyond the analytical capabilities of the methods employed in the present study and, therefore, it will be our future interest to look into this aspect in detail.

The drop in abundance of *Synechococcus* and nanoplankton observed during the Monsoon 1 sampling is closely linked to the rapid transformation of the Pre-Southwest Monsoon in hydrography to the Southwest Monsoon conditions due to heavy rainfall. The dilution effect induced by heavy rainfall and faster flushing of the Cochin backwaters during the Southwest Monsoon could also cause a decrease in the abundance of the plankton components (Wellershaus 1974; Jyothibabu et al. 2014). Even though the abundance of *Synechococcus* diminished during the Southwest Monsoon period, the total abundance of the picoplankton stock was relatively stable due to a corresponding increase in the pico - eukaryotes during the same period. *Prochlorococcus* was found to be either very low or absent in the Cochin backwaters, which can be attributed to their preference for clear oceanic waters. High nutrient levels in the coastal and estuarine waters are reportedly unfavorable to the *Prochlorococcus* community (Mann et al. 2002; Worden and Binder 2003).

Earlier studies showed that autotrophic nanoplankton are the major primary producers in the Cochin backwaters, and in the present study, their abundance was higher upstream during the typical Southwest Monsoon hydrographical setting of Monsoon 2 and 3 sessions. The salinity overlaid Tri-plot clearly presents a generalized distribution ecology of the autotrophic pico- and nanoplankton in the Cochin backwaters (Figure 9). The Tri-plot showed that the total abundance of *Synechococcus* in the Cochin backwaters was significantly higher when polyhaline/mesohaline waters occurred in the system, and such conditions occurred during Pre-Monsoon conditions. On the other hand, higher

abundance of autotrophic pico-eukaryotes and nanoplankton were linked to oligohaline conditions with high concentrations of silicate and nitrate, which usually occurs during the Southwest Monsoon

5. Conclusion

The present study in the Cochin backwaters clearly showed that *Synechococcus* forms an important picoplankton community during the Pre-Southwest Monsoon period. The *Synechococcus* community showed differences in their phycoerythrin and phycocyanin contents in the upstream and downstream regions. The phycoerythrin-rich cells were found to be abundant in the lower estuary (fluoresces yellow-orange color) while phycocyanin-rich cells (fluoresces red colour) were observed in the upper estuary. During the south west monsoon season, the cell abundance of *Synechococcus* diminished and the entire system was in the limnetic condition. *Synechococcus* observed during the south west monsoon period was the phycocyanin-rich red colored fresh water strain. During Pre- Southwest Monsoon, the grazer population was high, which consumed a major portion of primary producers. Previous studies have reported that herbivorous mesozooplankton and microzooplankton graze on micro- and nano-phytoplankton, but recent studies have shown that the picoplankton is grazed as well. It is necessary to include the role of autotrophic pico and nano phytoplankton in carbon transport because mesozooplankton plays a major role in indirect transport of the primary carbon to higher trophic levels.

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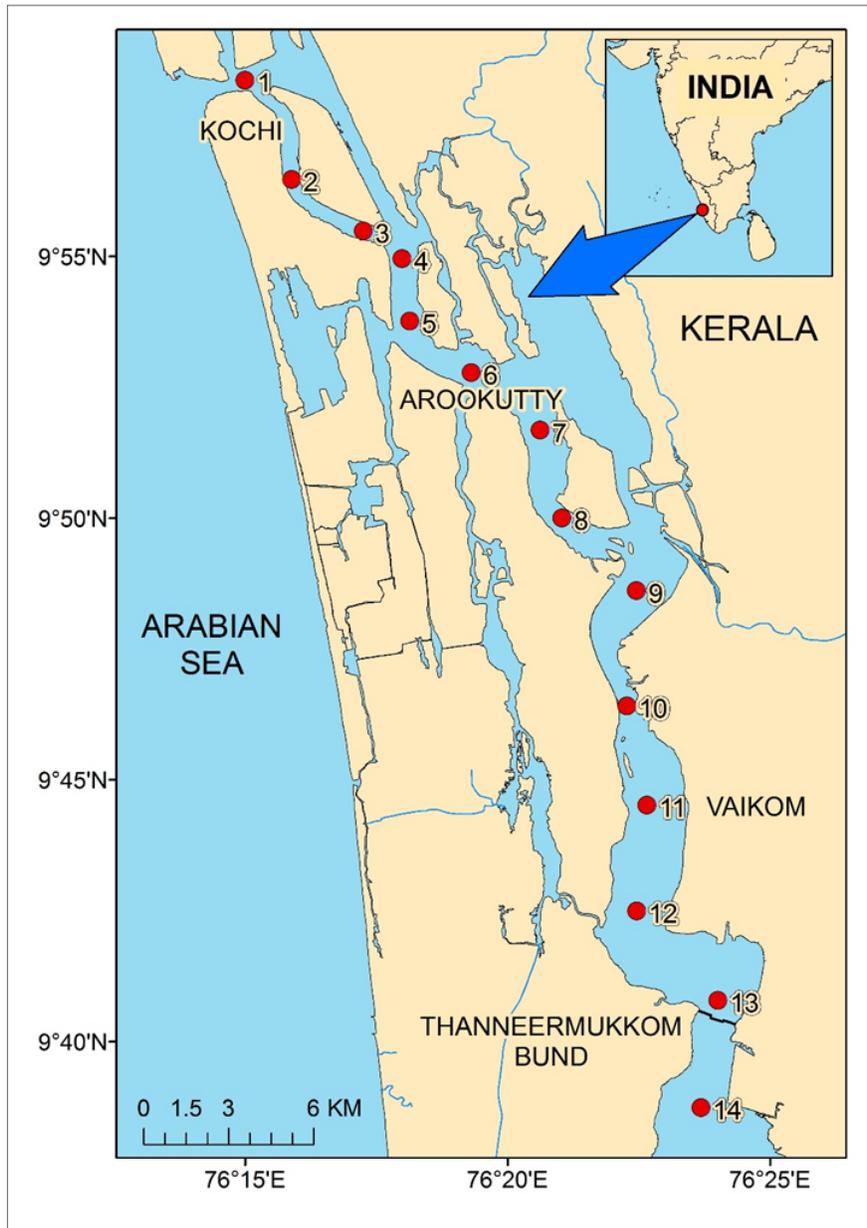


Figure 1 - Study locations (1-14) in the Cochin backwaters in 2011. The Pre-Monsoon sampling was on May 26, Southwest Monsoon sampling first on June 6 (Monsoon 1), the second on June 12 (Monsoon2) and the third on June 18 (Monsoon 3).

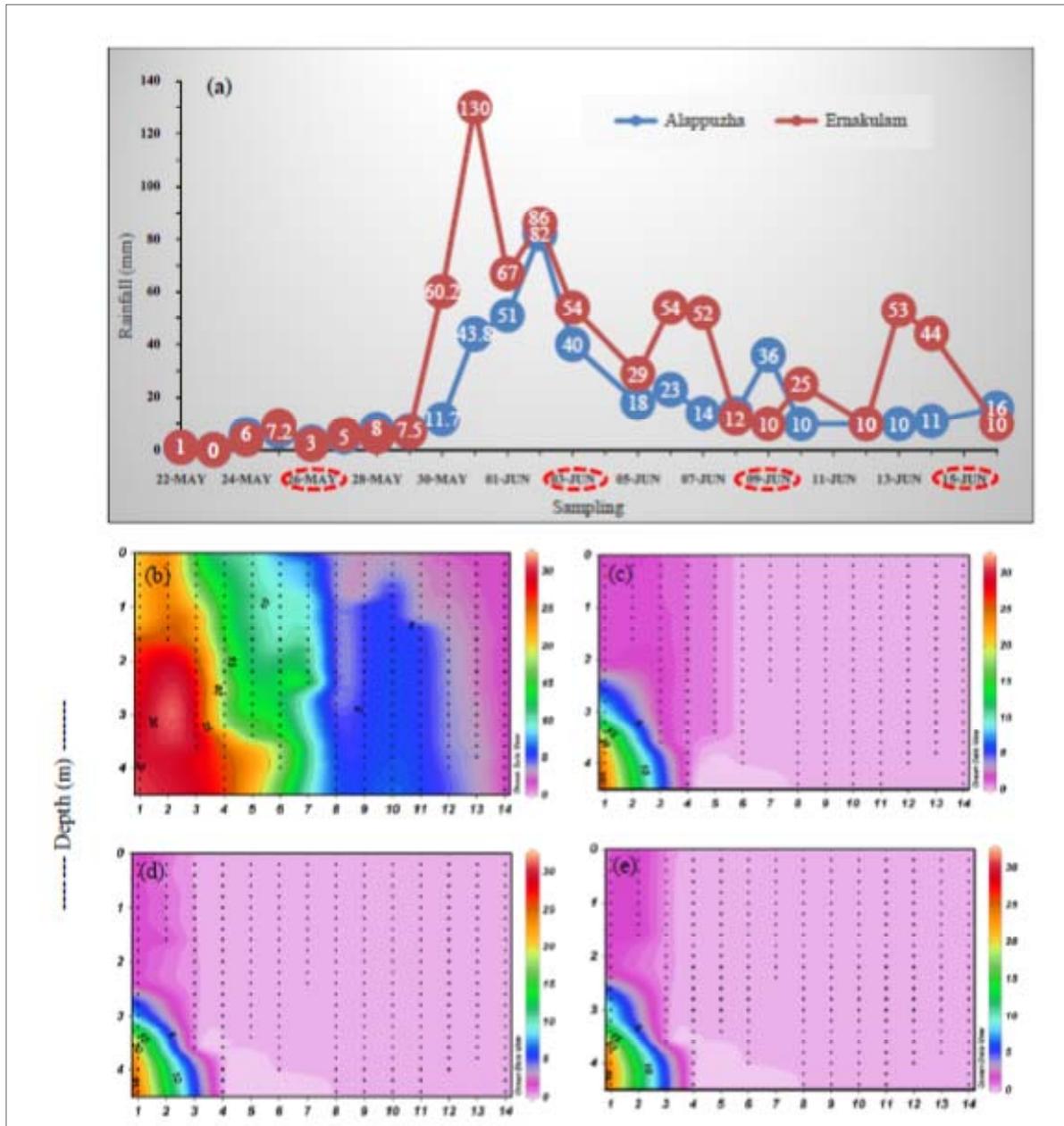


Figure 2 – (a) Rainfall in the study domain during the sampling period. The sampling dates are indicated in red dotted circles. Pre-monsoon sampling was on May 26, first Monsoon sampling on June 3, second Monsoon sampling on June 9 and the third Monsoon sampling on June 15 of 2011. Distribution of salinity based on CTD during (b) Pre - Southwest Monsoon (Pre-Monsoon), (c) onset of the Southwest Monsoon (Monsoon 1) and (d & e) Southwest Monsoon (Monsoon 2 and 3).

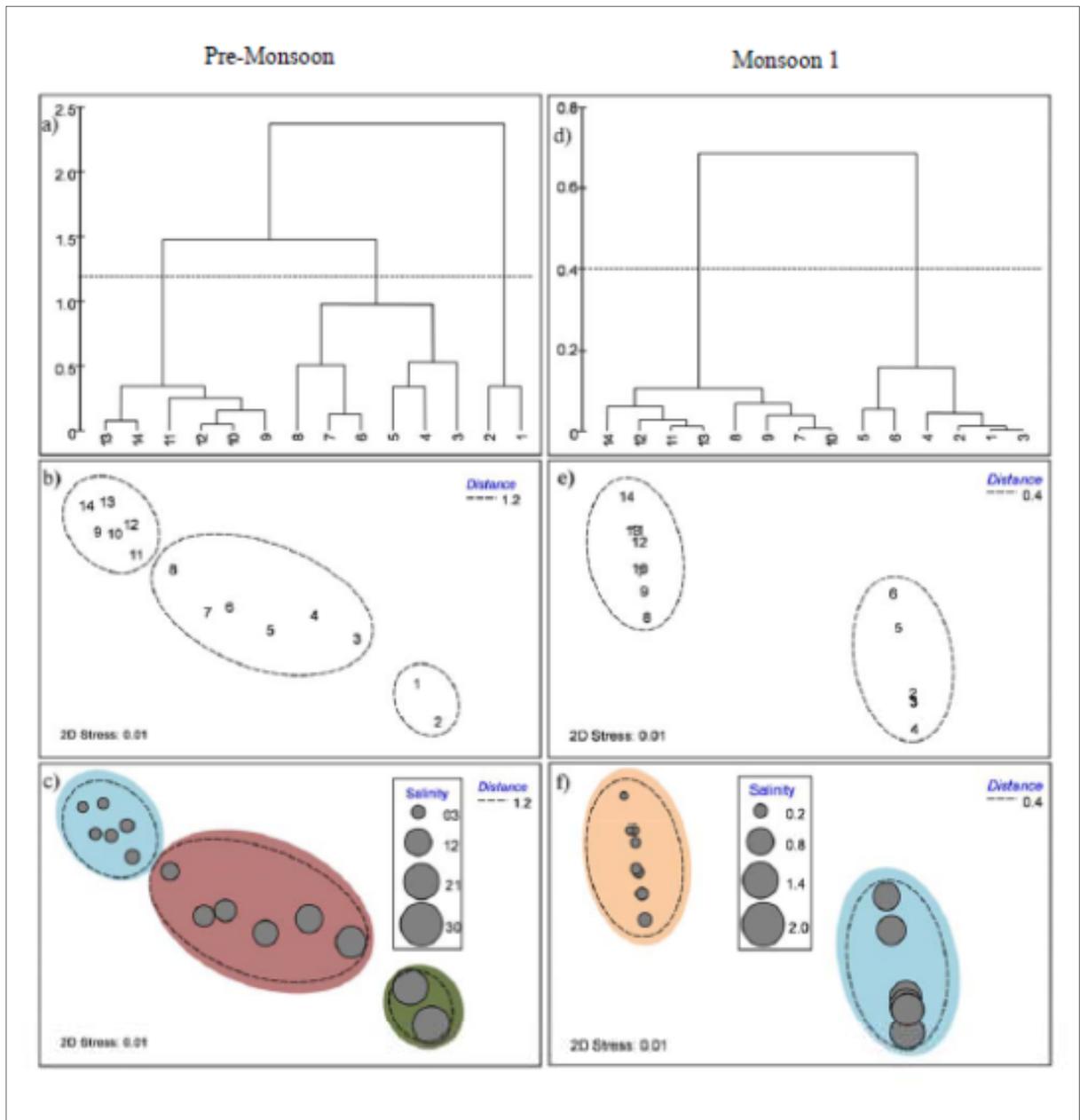


Figure 3 - Euclidian distance matrix based cluster dendrogram and NMDS plots representing (a-c) Pre-Monsoon and (d-f) onset of Southwest Monsoon (Monsoon1). Panels (c) and (d) represent salinity overlaid bubble plots on NMDS segregated locations during the Pre- monsoon and Onset of Southwest Monsoon respectively.

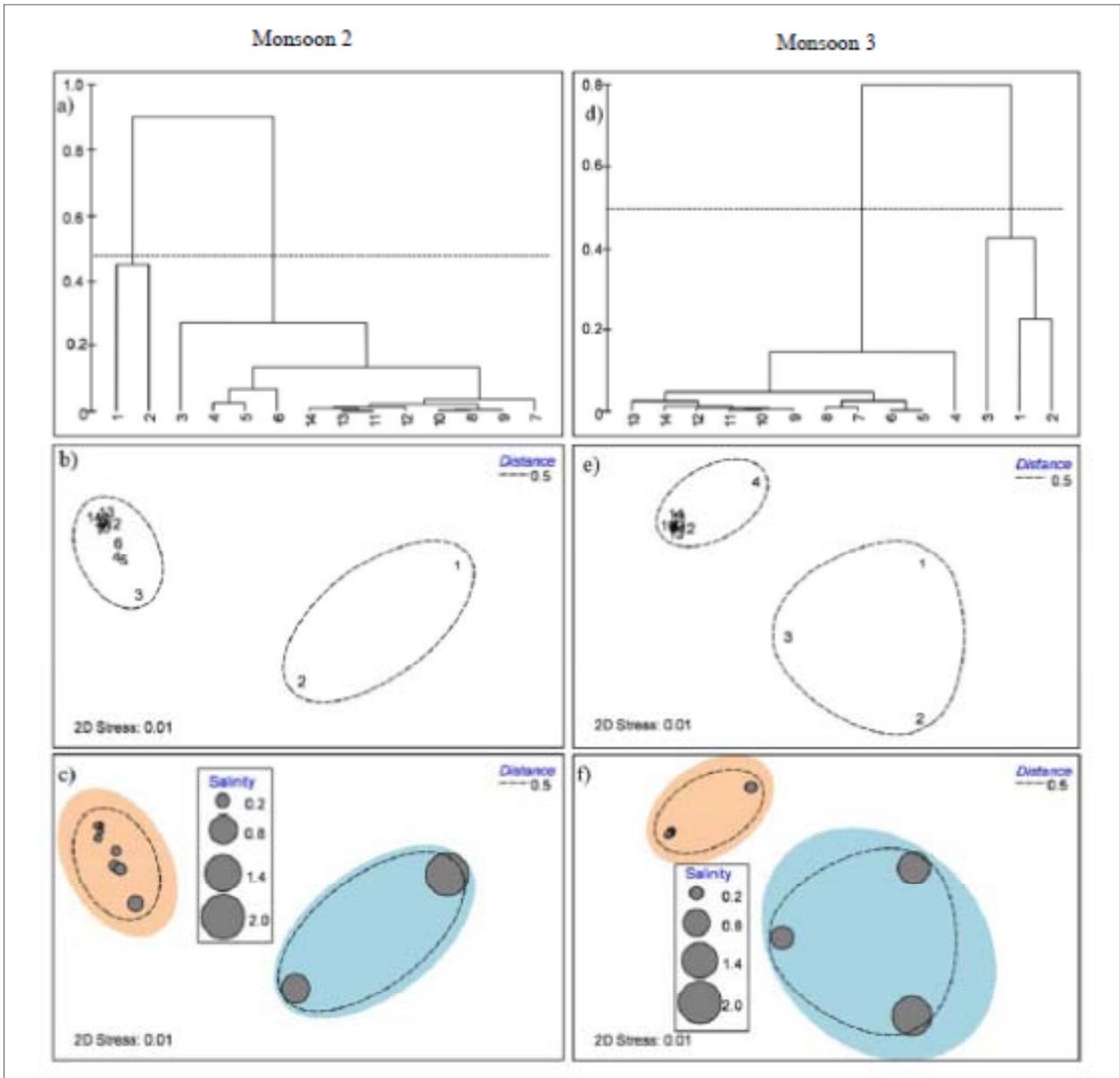


Figure 4 - Euclidian distance matrix based cluster dendrogram and NMDS plots representing (a-c) Monsoon 2 and (d-f) Monsoon 3. Panels (c) and (d) represent salinity overlaid bubble plots on NMDS segregated locations during the Monsoon 2 and Monsoon 3, respectively.

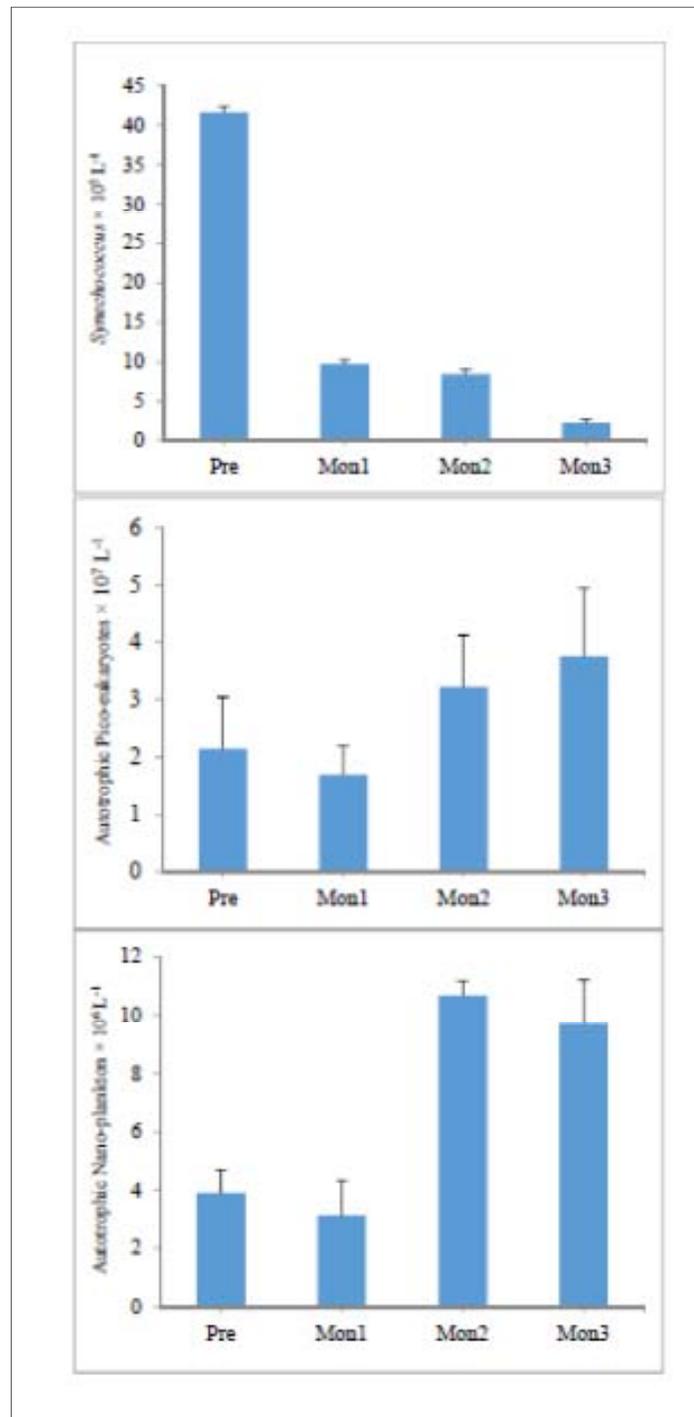


Figure 5 – Response of autotrophic pico- and nano-plankton to Southwest Monsoon

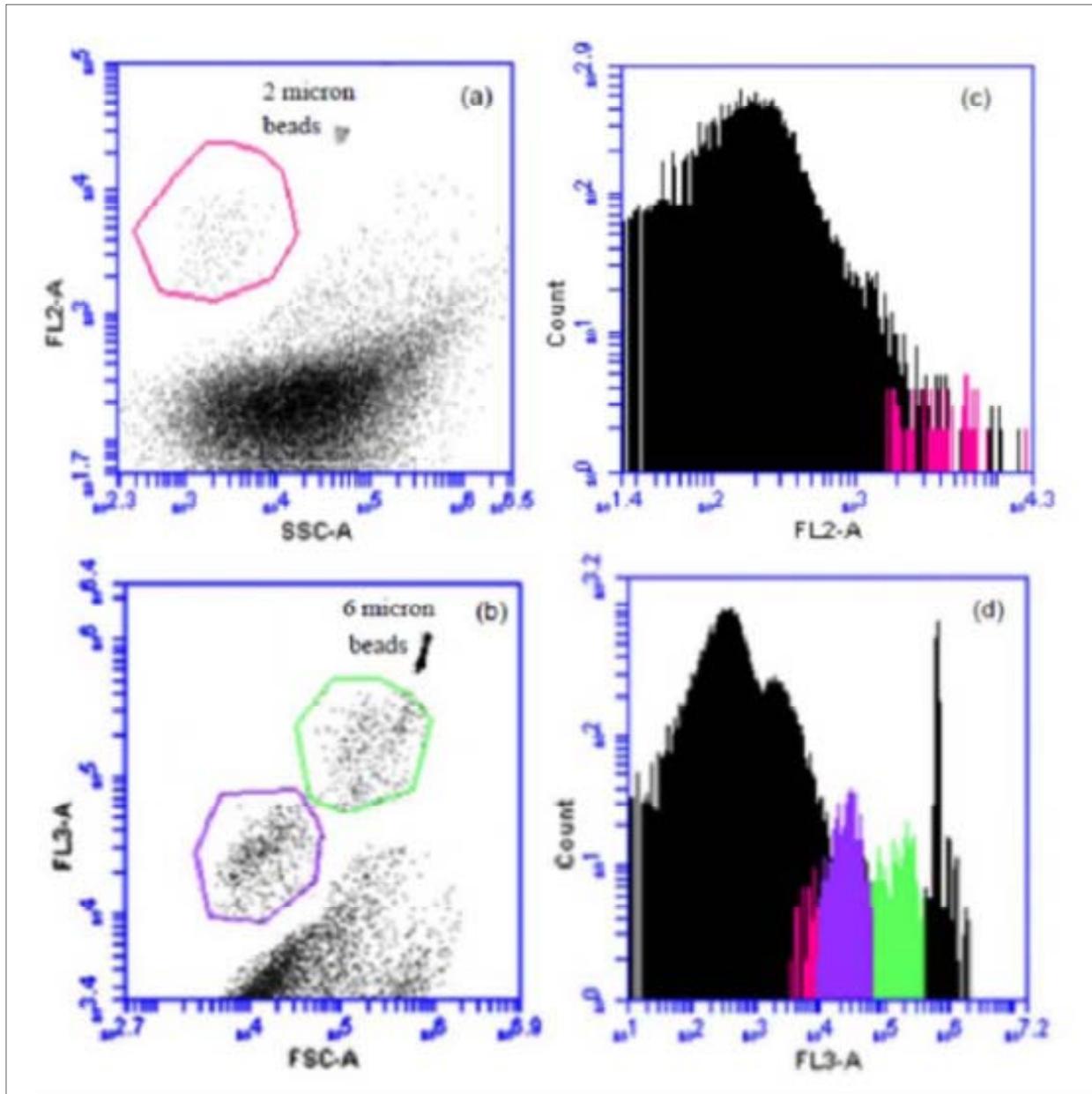


Figure 6 – Flow Cytometry (a & b) scatter plots and (b & c) fluorescence pattern of autotrophic pico - and nano - plankton in the Cochin backwaters. The size of (a) *Synechococcus* (purple circle) calibrated with 2 μ m beads and (b) pico-eukaryotes (violet circle) and nano-plankton (green circle) calibrated with 6 μ m beads. The histogram (c) shows the fluorescence of *Synechococcus* phycocyanin (purple colour) and (d) shows chlorophyll fluorescence of pico-eukaryotes (violet colour), nano-plankton (green colour) and *Synechococcus* (purple colour).

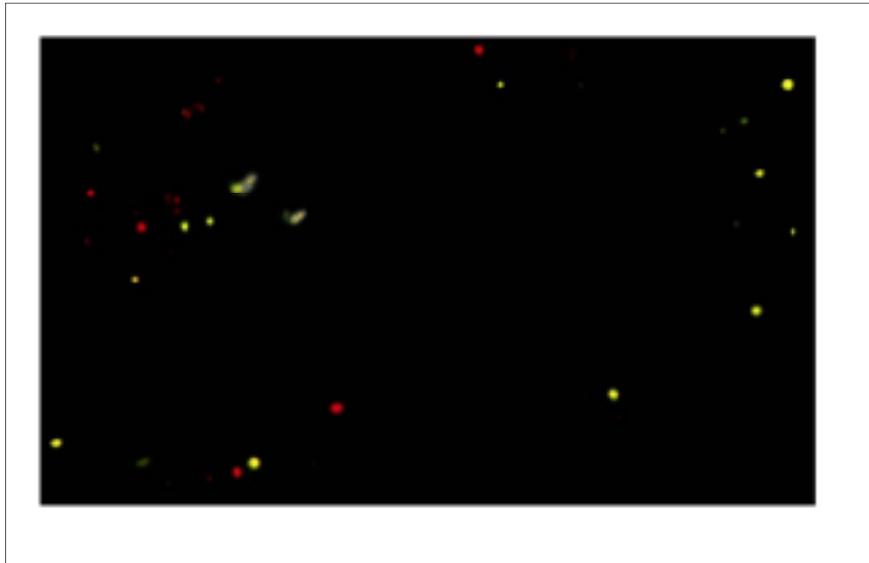


Figure 7 - Epifluorescence images of two strains of *Synechococcus* observed in different salinity regimes. The dominant phycoerythrin rich strain in the euhaline/ mesohaline was yellow/orange coloured whereas the dominant phycocyanin- rich strain in limnetic conditions was red coloured.

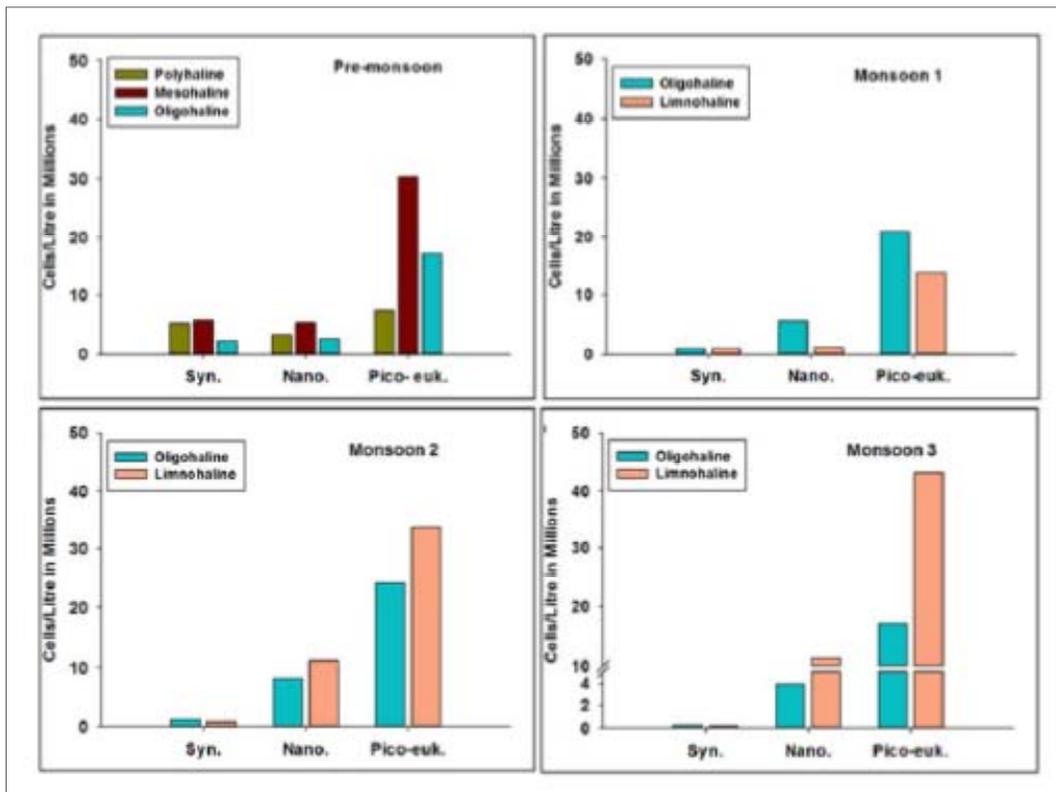


Figure 8 – Autotrophic pico – and nanoplankton in different salinity regimes

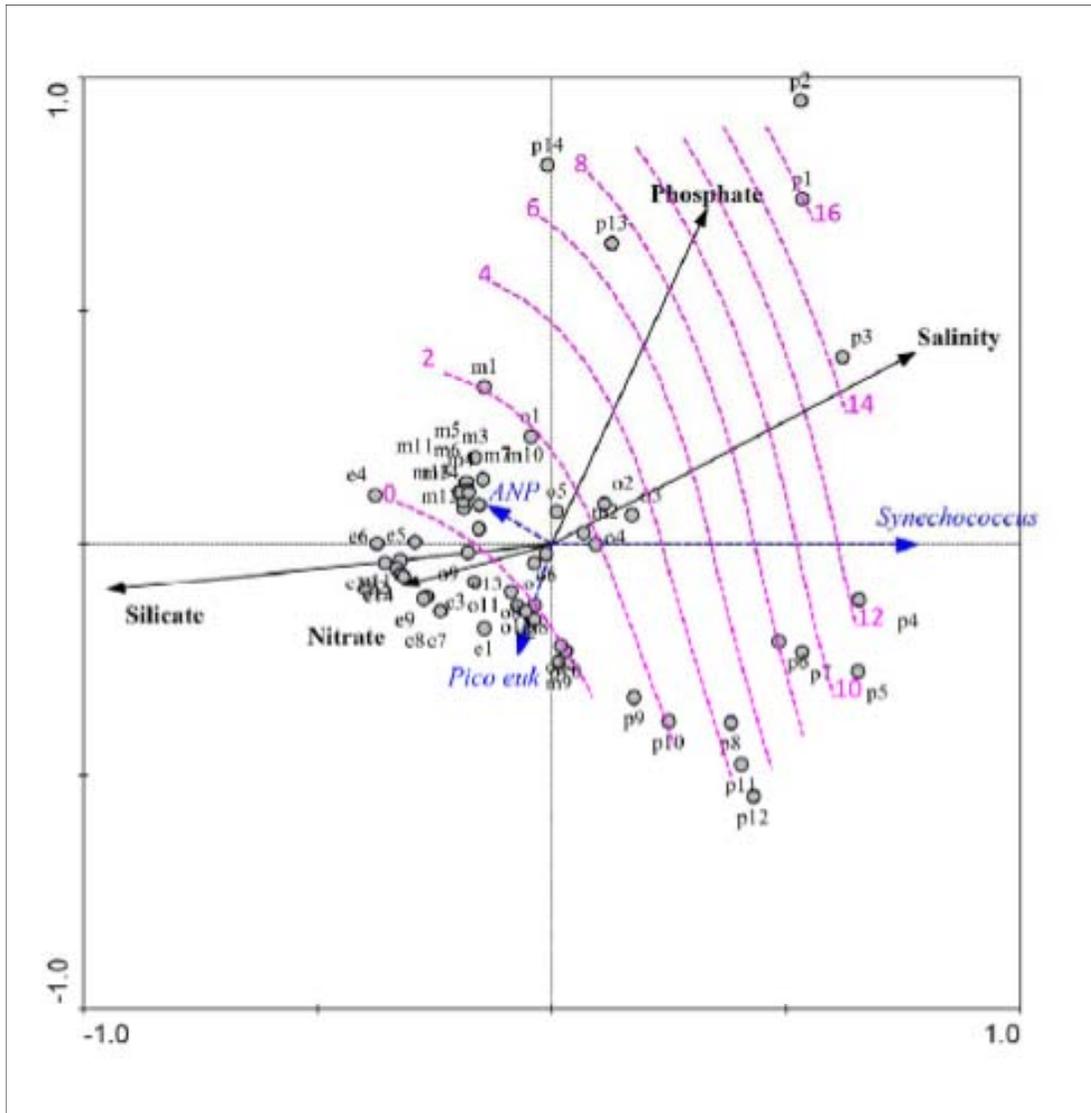


Figure 9 - Salinity contour overlaid (Pink dotted lines) triplot showing the inter relationships physico chemical parameters on distribution of Syn, Nano and Pico euk. In the plots filled circles represents the stations. In fig. p1-p14 represents the pre-monsoon collection and the sampling stations 1-14; o1-o14 represents the mon1 collection and the sampling stations 1-14; m1-m14 represents the mon2 and their sampling stations; e1-e14 represents the mon3 and their sampling stations. Syn-Synechococcus, ANP – Autotrophic nanoplankton, Pico euk – Pico eukaryotes.

Sampling	SiO ₄ (μ M)	NO ₃ (μ M)	PO ₄ (μ M)	Sal. (psu)	Tur. (NTU)	Chl <i>a</i> (mg m ⁻³)	Syn. ($\times 10^6$ L ⁻¹)	Pic – euk. ($\times 10^7$ L ⁻¹)	ANP ($\times 10^6$ L ⁻¹)
Pre-Mon.	38.6 (0.2)	20.3 (0.6)	0.23 (0.5)	8.4 (0.9)	5.3 (0.5)	4.8 (0.6)	41.5 (0.8)	2.1 (0.9)	3.8 (0.8)
Mon. 1	111.9 (0.1)	9.4 (0.5)	0.2 (0.5)	0.5 (0.9)	40.7 (0.8)	12.6 (0.6)	9.7 (0.6)	1.6 (0.5)	3.1 (1.2)
Mon. 2	117.5 (0.1)	16.9 (0.1)	0.3 (0.3)	0.3 (1.9)	14.9 (0.6)	2.7 (0.3)	8.5 (0.5)	3.2 (0.9)	10.6 (0.5)
Mon. 3	128.5 (0.1)	27.8 (0.2)	0.1 (0.6)	0.3 (1.7)	24.2 (1.1)	1.7 (0.8)	2.2 (0.4)	3.8 (1.2)	9.7 (1.5)

Table 1 - Parameters measured during the sampling in the Cochin backwaters. The mean values and coefficient of variations (in parenthesis).