

Response of smaller plankton to the saltwater barrage-altered hydrography of a tropical estuarine system on India's southwest coast

Arunpandi, N¹, *Jyothibabu, R¹, Jagadeesan, L^{1,2}, Arya, P.M¹, Rashid, C.P¹, Sarath, S¹

¹CSIR- National Institute of Oceanography, Regional Centre, Kochi, India

²CSIR- National Institute of Oceanography, Regional Centre, Vishakhapatnam, India

*Corresponding author: rjyothibabu@nio.org

Phone +91 (0) 484 2390814, Fax +91 (0) 484 2390618

Abstract

This study examines how a large saltwater barrage [Thannermukkom barrage (TB)] in the Kochi backwaters (KBW), India's largest estuarine system on the west coast, affects the distribution of autotrophic small plankton communities (*Synechococcus*, picoeukaryotes, and nanoplankton). The KBW has two distinct regions when the shutters of TB are closed during non-monsoon periods: a mesohaline to euhaline northern region downstream and a limnohaline southern region upstream. The current seasonal salinity distribution in the KBW revealed a significant decrease in salinity compared to that before the establishment of TB. Considering this, we hypothesised that the human-induced hydrographical transformation associated with the functioning of TB has implications for the ecology of the smaller plankton components in the system, and to test this, smaller autotrophic plankton and hydrographical variables were collected from the KBW during three different seasons, including the Pre-Monsoon [(PRM) March-May], the Southwest Monsoon (SWM) from June to September, and the Northeast Monsoon (NEM) from November to February. Seasonally, 13 locations along the KBW were sampled, with locations 1-4 in the downstream, 5-9 in the midstream, and 10-13 in the upstream. The findings revealed that, regardless of season, a mesohaline condition in the KBW is the most conducive one that sustains an increased abundance of autotrophic pico- and nano-plankton communities. Two strains of *Synechococcus* (SYN) [phycoerythrin rich SYN I and phycocyanin rich SYN II] contribute nearly equally in the mesohaline region, whereas a predominance of SYN II (>90%) was found in oligohaline/limnohaline conditions. We show that TB reduces the geographic extent of the mesohaline zone in the KBW that is most conducive to maintaining a high abundance of smaller plankton communities, reducing the total abundance and biomass of the smaller plankton community in the system.

Keywords: plankton, distribution, barrage, salinity, Kochi backwaters, India

1. Introduction

Smaller autotrophic plankton [pico- (0.2–2 μ m) and nano-(2–20 μ m)] are found in aquatic systems all over the world, which include both prokaryotes and eukaryotes (Veldhuis et al., 1993; Campbell et al., 1997). *Prochlorococcus*, *Synechococcus*, and picoeukaryotes are the most abundant autotrophic picoplankton (APP) (Partensky et al., 1996), with the former being more abundant in oceanic waters and the latter in coastal and estuarine environments (Mitbavkar and Anil, 2011). In estuarine and marine situations, autotrophic nanoplankton (ANP) constitutes the majority of phytoplankton biomass and is the primary source of dissolved organic matter (Lugioyo et al., 2007; Pan et al., 2007; Tarran et al., 2001; Madhu et al., 2010). Smaller plankton is important for primary production and the aquatic carbon cycle (Li, 1994; Worden et al., 2004), accounting for more than half of gross primary production in oligotrophic waters (Li et al., 1983; Stockner and Anita, 1986). Despite being smaller in size than the other plankton functional groups, picophytoplankton play an important ecological role in transferring carbon to higher trophic levels, especially in oligotrophic conditions (Richardson and Jackson, 2007). Interestingly, autotrophic picoplankton is more abundant in nutrient-rich coastal and estuarine waters, despite contributing significantly less to gross primary production than larger phytoplankton (Mitbavkar and Anil, 2011; Mitbavkar et al., 2012).

The use of fluorescent microscopy and flow cytometry allowed researchers to quantify the smaller plankton communities found in marine and estuarine systems (Burkill et al., 1993; Landry et al., 1996; Garrison et al., 2000; Brown et al., 2002; Jyothibabu et al., 2013; Arya et al., 2016). Because of the freshwater (limnetic) conditions at the river end and seawater incursion at the other end, the estuaries are productive with spatially varying hydrographical settings (Haridevan et al., 2015; Arunpandi et al., 2020a,b). As human settlements expand along estuary banks, the hydrographic modifications of the natural systems are becoming more common around the world to meet a variety of human needs. Human alterations of the natural aquatic systems and the ways they influence biodiversity and the trophic status of the system are important research aspects considered worldwide (Ehrlich and Wilson, 1991; Chapin et al., 2000). As wetlands are always sensitive ecosystems, environmentalists have great concern over anthropogenic alterations of such systems and their implications for the life existing in them as well as their ramifications for the local community living in their proximity (Huston, 1979). Although there are several factors which could influence the plankton community in an estuary, past studies have evidenced salinity as the most important environmental factor controlling the distribution of biota in estuaries (Haridevan et al., 2015; Arunpandi et al., 2021a, b) and a significant drop in salinity has a great potential to drastically change the prevailing smaller plankton community (Arya et al., 2016). Even though there are numerous estuaries located along the Indian coastline, peer-reviewed

literature on the impact of man-made salinity barrages on the hydrology and living resources in Indian estuaries is still very limited (Gopalan et al., 1983; Balchand, 1983; Achari, 1988; Froneman, 2004; Arun, 2005; Haridevan et al., 2015). Although these barrages serve some human needs, they adversely affect the quality of the natural environment and result in the loss of many valuable biological resources (Gopalan et al., 1983; Balchand, 1983; Haridevan et al., 2015; Arunpandi et al., 2020a,b; 2021a,b; Rashid et al., 2022).

The Kochi backwaters (KBW) is the largest monsoonal estuary along the west coast of India, receiving a massive freshwater discharge from six rivers as well as salinity incursion from the Arabian Sea through periodic tidal action. The Thanneermukkam barrage (TB), a saltwater barrage, was built across the KBW in 1976 (Arunpandi et al., 2020a,b; Arunpandi et al., 2021a,b). The TB divides KBW into two sections during non-monsoon periods: the limnetic upstream region and the mesohaline to euhaline downstream region (Haridevan et al., 2015; Anjusha et al., 2018; Arunpandi et al., 2020a; Arunpandi et al., 2021b). TB has been shown in the past to affect the system's hydrobiology and disturb the migration paths of living resources like prawns, fish, and clams (Padmakumar et al., 2002, Arun, 2005). Whatever the case, changes in salinity reduce plankton growth and diversity (Sugie et al., 2020), and decreasing salinity in the upstream region owing to TB has an impact on the plankton food web components in the KBW (Gopalan et al., 1983; Anjusha et al., 2018; Arunpandi et al., 2020a,b; Arunpandi et al., 2021a, b).

Pico and nanoplankton play an essential role over the entire stretch of the KBW, although their abundance varies according to salinity gradients (Arya et al., 2016). Pico-cyanobacteria (*Synechococcus*) have shown many physiological adaptations to combat salinity stress, including the buildup of suitable salts (an intracellular mechanism) and the creation of exopolymeric compounds (extracellular method) (Brown, 1976; Qiu et al., 2012; Bemal and Anil, 2018; Kumar et al., 2021). Plankton cell development, physiology, and cell division are all affected by salinity stress. Even though smaller plankton displayed a variety of adaptation strategies to prevent salt stress, their tolerance limits varied depending on the strain (Bemal and Anil, 2018; Rezayian et al., 2017). Rezayian et al., (2017) and Kumar et al., (2021) pointed out that the smaller variations of the salinity also significantly affect the growth and production of smaller plankton. Also, there are some studies available on various plankton components of the KBW (Jyothibabu et al., 2006; Madhu et al., 2007; Haridevan et al., 2015; Arya et al., 2016; Anjusha et al., 2018; Arunpandi et al., 2020a, b; 2021a, b). However, there was no comprehensive information on the ecology of various groups of smaller plankton currently available and also how the salinity changes in the KBW impacted the smaller plankton components in the

system. So, the focus of this study is on how TB affected the smaller autotrophic plankton and how they responded to changing salinity levels.

Plankton is understood as a good indicator of environmental change around the world (Compos et al., 2017; Jagadeesan et al., 2013; Jyothibabu et al., 2018). Considering the large scale environmental modifications caused by the extensive TB, we hypothesize a possible alteration in the distribution of smaller plankton components in the KBW. To investigate this, we quantified all the smaller autotrophic plankton components in the KBW on a spatial scale during different seasons, which was used to infer how the TB altered hydrography impacted their natural distribution patterns. The primary objectives of this study are (a) to quantify the spatial variations of the smaller autotrophic plankton community in the entire KBW in different hydrographic settings and seasons, and (b) to investigate how the operation of TB altered the spatial distribution of smaller autotrophic plankton communities in the KBW.

2. Materials and methods

2.1. Study area

The KBW ($9^{\circ} 40'$ to $10^{\circ} 12'$ N and $76^{\circ} 10'$ to $76^{\circ} 30'$ E) is the largest monsoonal estuary along the southwest coast of India (Fig. 1). Salinity dominates the KBW during the non-monsoon period, while during the SWM, it receives a massive amount of freshwater from six rivers (Periyar, Achencovil, Pamba, Manimala, Meenachil, and Muvattupuzha) and at this time the salinity incursion is restricted only to its inlet region (Jyothibabu et al. 2006). The TB is constructed across the KBW in its upstream and the closure of the TB separates the KBW into two, a vast mesohaline to euhaline region in the downstream and a limnetic region in the upstream (Arunpandi et al., 2020a,b). To stop the saline water intrusion upstream, the shutters of TB are kept closed from December to May (Arun, 2005). TB is constructed to regulate the flow of saline water into the paddy fields in Kuttanad upstream (the Rice Bowl of Kerala) to help the farmers carry out multiple crops every year. Though the construction of the TB helped the farmers in improving the agriculture practices in Kuttanad, it created a lot of environmental problems as well, which is a serious matter of concern for the local administrators now. For the current study, the seasonal sampling was carried out during the Southwest Monsoon (SWM-June) and Northeast Monsoon (NEM-December) in 2013 and the Pre-Monsoon (PRM-March) in 2015 from 13 locations along the entire stretch of the KBW; 4 in the downstream (locations 1 to 4), 5 in the midstream (locations 5 to 9) and 4 in the upstream (locations 10 to 13) region (Fig. 1). The sampling locations were categorized into different salinity levels suggested by McLusky (1993);

euhaline (salinity >30) polyhaline (salinity 18-30); mesohaline (salinity 5-18); oligohaline (salinity 0.5-5); and limnohaline (salinity < 0.5).

2.2. Physico-chemical variables

The vertical distribution of salinity and temperature were recorded with a portable CTD profiler (Seabird- SBE 19plus V2) and the surface turbidity was measured by calibrated handy turbidity meter (Eutech TN-100), which functions as per nephelometric principles. Water samples for nutrients (silicate, nitrate and phosphate) and dissolved oxygen (DO) were collected from the surface layers using the 5L Niskin sampler (Hydrobios-Kiel). Nutrients were analysed based on the standard colourimetric method (Grasshoff et al., 1983) and DO was measured using Winkler's method. The historical data set of seasonal salinity distribution in the KBW before the construction of TB was adapted from Haridas et al., (1973) to compare with the current data.

2.3. Biological variables

Chlorophyll a (Chl-a) was used as a phytoplankton biomass index. For Chl-a analysis, one litre of the water samples was filtered through 0.2 µm millipore filter paper, which was placed in 10 ml of 90% acetone and incubated at 4°C in dark conditions for 24 hrs. Subsequently, the Chl-a content extracted in acetone was measured using a Trilogy fluorometer (Turner Designs, USA) following the standard procedure (UNESCO, 1994). To quantify the autotrophic pico and nanoplankton, 10ml of water samples were collected in cryovials and fixed with 1% glutaraldehyde and stored at -80°C (Vaultot et al., 1996; Jyothibabu et al., 2013; Arya et al., 2016). Pico-plankton (*Synechococcus* and picoeukaryotes) and nano-plankton were quantified using a flow cytometer (BD accuri C6). Initially, samples were passed through the 20 µm mesh to remove the larger particles before being loaded into 96 well plates (Jyothibabu et al., 2013; Arya et al., 2016). 100µl of samples were run at the medium speed of the instrumental setting and the autotrophic picoplankton were quantified based on their size and fluorescence properties (Chl-a, phycoerythrin and phycocyanin). During flow cytometric analysis, the flowcytogram provides two parameters scatter plots that indicate distinct clusters of particles of the same or comparable properties (Moreira-Turcq and Martin, 1998). In the present study, *Prochlorococcus* was omitted because of the non-detection of *Prochlorococcus* in the sample.

Water samples (20 ml) were collected and carefully transferred into sterilised dark polyethene bottles, kept in 1% final concentration of glutaraldehyde, and maintained in an icebox for imaging of autotrophic picoplankton under the microscope (Jyothibabu et al., 2013; Arya et al., 2016). To eliminate larger particles, the samples (10 ml) were pre-filtered in the lab via sterile filters with a pore size of 3 µm. (Porter and Feig 1980). The samples were then filtered using 0.2 µm black nucleopore filters

(25 mm diameter) and placed in immersion oil for analysis under blue excitation using an Olympus BX 53 epifluorescence microscope with an image analyzer (progRes Capture Pro 2.6). The microscopic analysis was carried out as soon as the slide was prepared (Bloem et al., 1986). For visualization of autotrophic nanoplankton under the microscope, 20ml water samples preserved in glutaraldehyde were filtered through a 20 μm mesh to remove the larger particles in the sample and the filtrate was stained with 1.65 $\mu\text{g ml}^{-1}$ proflavin hemisulfate and filtered through a 0.8 μm pore sized Nucleopore filter (Haas, 1982). This filter is mounted in immersion oil and analyzed under an epifluorescence microscope. It fluoresced green under blue illumination and autotrophic nanoplankton was taken using the image analyzer.

2.4. Statistical analysis

A cluster NMDS (Euclidian distance matrix) analysis was performed for grouping the sampling locations based on their similarity in hydrographical variables. Initially, hydrographical variables were normalized before the analysis. NMDS analysis was performed separately for the three seasons. The locations within the cluster have more similarities, whereas there is a dissimilarity between locations in different clusters. The significance of the cluster pattern was performed through SIMPROF permutation analysis (Leps and Smilauer, 2003). TB induced hydrographical changes and pico and nanoplankton were tested using ANOVA. Firstly, the arranged datasets were tested for their distribution pattern. Then the homogenous datasets were treated with normal distribution parametric ANOVA through Tukey's HSD posthoc test to compare the variance between the clusters. The heterogeneous sample distribution data was treated with Non-parametric ANOVA (Kruskal- Wallis) with Dunns post hoc test for comparing the variances between the clusters. These ANOVA tests were carried out in the XL stat pro-software package. The interrelationships within and between the physical-chemical and the biological variables [Chl-*a*, APP (*Synechococcus*, Picoeukaryotes) and ANP (autotrophic nanoplankton)] were analysed with RDA (CANOCO 4.5). Initially, the data was tested with Detrended Correspondence Analysis to find a suitable ordination method. The Detrended Correspondence Analysis results showed an axis gradient length < 2, suggesting the use of a linear multivariate RDA as a suitable technique (Leps and Smilauer, 2003). The abundance data of smaller plankton (APP and ANP) was log-transformed before the analysis. The ordination significance was tested with Monte Carlo permutation tests (499 unrestricted permutations) ($p < 0.05$). The results of the RDA analysis have been presented in Triplots.

3. Results

3.1. General hydrographical settings

The salinity distribution in the KBW is shown in Fig. 2. During the PRM when TB was closed, the downstream and midstream of KBW had polyhaline to mesohaline waters with a surface salinity from 0.89 to 26.4 (Fig. 2a and Table 1). Polyhaline salinity (26.4) was observed in the downstream (station 1) and mesohaline in the midstream. Almost 5 unit salinity difference was noticed between the midstream and upstream regions in the KBW during the PRM. The tidal input of salinity was mostly limited to the southern limit of the midstream region (station 9). And in the upstream region in the south of TB in the KBW had oligohaline to limnetic levels of salinity (0.89 - 4.9) during the PRM.

During the SWM, when TB was open, the entire KBW had oligohaline to limnetic conditions except in the extreme downstream region closer to the inlet, where polyhaline salinity was found (Fig. 2b). During the NEM, when the TB was partially closed, salinity in the KBW ranged from mesohaline to limnetic (Fig. 2c). The downstream zone was found to be mesohaline, whereas the upstream region was found to be oligohaline, with a 2 unit salinity difference on both sides of the TB (Table 1). A comparison of current data with historical data before the operation of TB was utilised to investigate how TB impacted the hydrographic setting of KBW (Fig. 3). The TB reduced salinity levels in the KBW during all seasons, but especially during the SWM. In effect, TB considerably decreased the spread of mesohaline conditions in the midstream and upstream regions of the KBW during different seasons.

Seasonally, DO was high throughout the KBW with the peak during the SWM. The upstream of the KBW showed a clear increase in DO regardless of seasons (Table 1). Turbidity was high in downstream than in the midstream and the upstream locations and seasonally it varied with the highest values during the SWM followed by the NEM and the PRM (Table 1). Spatial variations in nutrients are presented in Fig. 4. The concentration of nitrate (NO_3) varied between 0.63 and 18.53 μM in KBW. The spatial distribution of NO_3 did not show any clear pattern during the SWM. But its higher concentration was found in upstream t in downstream during the PRM and NEM. Phosphate (PO_4) concentrations varied from 0.13 to 2.05 μM in the KBW (Fig. 4). PO_4 concentration in the KBW was found to be lower during the SWM than in the PRM. Seasonally, PO_4 concentration was higher during the NEM followed by the SWM and the PRM. Spatially, PO_4 was high downstream regardless of seasons (Table 1). During the PRM, silicate (SiO_4) concentration varied from 13 to 49.1 μM (Fig. 4a). SiO_4 was found remarkably higher in the entire KBW during the SWM (5 times) compared to the PRM and the NEM. The total Chl-*a* in the KBW is presented in Fig. 5. During the PRM, Chl-*a* varied from

1.55 to 9.22 mg.m⁻³, the SWM from 1.11 to 4.2 mg.m⁻³ and the NEM from 1.48 to 6.61 mg.m⁻³. The highest and the lowest Chl-*a* were found upstream during the PRM and the SWM, respectively (Fig. 5). The Chl-*a* concentration in the KBW was found to be high (>1 mg m⁻³) irrespective of seasons and regions.

3.2. Clustering the locations

The sampling locations were grouped based on the salinity levels. During the PRM, four clusters of locations were found; locations 1 and 2 in cluster I [polyhaline (av. 23.7 ± 3.8)], 3 to 6 in cluster II [mesohaline (av. 14.5 ± 2.1)], 7-9 in cluster III [mesohaline (av. 10.46 ± 0.53)] and locations 10-13 in cluster IV [oligohaline (av. 2 ± 1.9)] (Fig. 6a). During the SWM, spatial salinity variations in the KBW were too low, that the locations were grouped as a single cluster except location 1 in the inlet region downstream, representing that the entire KBW was dominated by freshwater (limnohaline <0.5 salinity) (Fig. 6c). During the NEM, the study locations were grouped into three clusters: locations 1 - 3 in cluster I [mesohaline (av. 14.3 ± 2.1 salinity)], locations 4-9 in cluster II characterized as [mesohaline to oligohaline (av. 7.58 ± 2.02)]; and locations 10-13 in the upstream [oligohaline region (av. 0.26 ± 0.25)] grouped into cluster III (Fig. 6e). The spatial comparison (ANOVA) of hydrographical and biological parameters in different seasons revealed that PRM and NEM differed significantly (p<0.05) from the SWM (Table 2).

3.3. Autotrophic picoplankton (APP)

From the results of flow cytometric analysis, *Synechococcus* and picoeukaryotes were the major contributors to the APP community in the KBW. The total APP abundance in the study locations varied from 0.56 - 4.01 (x10⁷) cells L⁻¹ (Table 1). APP abundance and their proportionate spatial differences during various periods are represented as overlaid bubbles in NMDS plots (Fig. 6b, d and f). The photomicrographs of APP under the fluorescent microscope are presented in Supplemental Material 1. Total APP abundance was high during the PRM, and it decreased considerably during the SWM. Regardless of locations, the abundance of picoeukaryotes was found to be higher than *Synechococcus* and contributed 52.3 % to 99.56 % of the total APP abundance. The contribution of Picoeukaryotes to the total picoplankton community abundance was found higher upstream regardless of seasons. In the current study, two *Synechococcus* strains were found: phycoerythrin (PE)-rich (SYN I) and phycocyanin (PC)-rich (SYN II).

The distribution of *Synechococcus* strains differed spatially and seasonally in the KBW (Fig. 7). During the PRM (TB shutters closed), the total abundance of APP was higher in cluster II of mesohaline midstream locations [1.87 - 4.01 (x10⁷) cells L⁻¹] compared to other clusters (Fig. 6b and

Table 1). The total APP in cluster I (downstream), cluster III (midstream near to TB) and cluster IV (upstream) were comparable in counts, but the relative abundance of *Synechococcus* and picoeukaryotes varied in these clusters (Fig. 7). In the downstream (cluster I), *Synechococcus* contributed significantly (30-40%) to the total abundance; whereas, in cluster III (locations 7 - 9) and cluster IV (locations 10 - 13), they contributed only 4-7% of the total APP. The contribution of picoeukaryotes was dominant in cluster III and cluster IV locations. The APP abundance was higher in the midstream and downstream locations than in the upstream locations (Fig. 6b). SYNI strain contributed 90% of the total *Synechococcus* abundance in cluster I (polyhaline downstream). In cluster II in the mesohaline regions, both strains of SYN (SYN I and SYN II) had contributed almost equal proportions. In cluster III (locations 7 - 9), SYNI and SYNII were found, but the latter contributed 80% of the total abundance (Fig. 7a). In cluster IV in the oligohaline region, only SYNII was found while SYNI was completely absent there.

During the SWM (TB shutters open), APP abundance was lower ($1.14 = 4.01 \times 10^7$ cells L⁻¹) in the entire stretch of KBW compared to the PRM (Fig. 6d). Spatially, *Synechococcus* abundance was higher in cluster I location in the inlet, with low abundance in all other locations with low spatial variations. The abundance of picoeukaryotes was relatively high in upstream locations, with low spatial differences. The SYN II strain contributed >90% abundance even in the downstream locations (Fig. 7b). During the NEM (TB partially closed), total APP abundance varied from 1.16 - 2.12 ($\times 10^7$) cells L⁻¹ (Table 1 and Fig. 6f). The total abundance of APP did not vary significantly between locations even if the contributions of *Synechococcus* and picoeukaryotes varied spatially (Fig. 7c). *Synechococcus* abundance was higher in the downstream compared to the upstream, while an opposite spatial trend was shown by picoeukaryotes. SYN I and SYN II were found downstream with the former in higher abundance (Fig. 7c). In the midstream and upstream, SYNI was less abundant and it was nearly absent in the upstream locations.

3.4. Autotrophic nanoplankton (ANP)

The abundance of ANP in the study region ranged from 0.65 - 10.08 ($\times 10^6$) cells L⁻¹ (Fig. 8 and Table 1). Fluorescence images of ANP from the study region are presented in Supplementary Material 2. The spatial distribution pattern of ANP varied between seasons. During the PRM, ANP abundance was higher in cluster II mesohaline midstream locations [$8 - 10.08 (\times 10^6)$ cells L⁻¹], which was 2 to 3 folds higher than in cluster I polyhaline downstream and cluster IV oligohaline upstream (Fig. 8b). During the SWM, ANP abundance was low in the entire study area and varied from 0.65 - 1.83 ($\times 10^6$) cells L⁻¹ (Table 1). Spatially, ANP was found higher in the downstream (cluster I) compared to the upstream (cluster II) locations in the study area (Fig. 8d). During the NEM, ANP abundance varied

from 1.84 - 5.96 ($\times 10^6$) cells L^{-1} (Fig. 8f). Spatially, ANP abundance was high in the limnohaline upstream compared to the downstream. The total ANP abundance in the downstream and midstream was comparable during the NEM. Noticeably, green microcystis bloom was found in the upstream region at that time (Supplemental material 2c).

3.5. Optimum salinity regime

To further understand the associations between hydrographic characteristics and the distribution of smaller plankton in the KBW, a multivariate RDA was performed. The RDA defined the geographic variation in hydrographical characteristics and their impact on the distribution of smaller plankton components in KBW during various sampling periods (Fig. 9). The downstream sites experienced excessive salinity and turbidity during the PRM. Salinity and turbidity, on the other hand, were low further upstream. The nitrate and silicate axes were orientated opposing the salinity, turbidity, and phosphate axes. The salinity overlay RDA map revealed that the downstream was polyhaline, the midstream was mesohaline, and the upstream was oligohaline to limnetic. The RDA triplot revealed a distinct pattern in the distributions of smaller plankton components. APP and *Synechococcus* (SYN) were orientated on the right side of the RDA plot during the PRM, and the salinity overlay revealed that their high abundance was in the mesohaline midstream area. Picoeukaryotes were orientated on the left side of the RDA plot during the PRM, indicating their abundance in the upstream oligohaline area. ANP distribution was discovered across the KBW throughout the time, although the salinity overlay suggests that their peak abundance was oriented towards the mesohaline midstream zone. During the PRM, the abundance of SYN and ANP was discovered to be greatest in the mesohaline midstream zone, and picoeukaryotes were found to be abundant in upstream sites (Fig. 9a).

During the SWM, the entire KBW was dominated by freshwater and there was no clear pattern in the distribution of APP and ANP. However, spatially, ANP and *Synechococcus* were found high the downstream, and picoeukaryotes the upstream. During the NEM, the spatial distribution of hydrographical parameters and smaller plankton components were defined in the RDA plot. Salinity and turbidity were high downstream and their increasing gradients were oriented to the right-hand side of the RDA plot. The salinity overlay showed that the downstream locations had mesohaline salinity, and the salinity decreased towards the upstream locations. During the NEM, APP and *Synechococcus* were found highly abundant downstream and were oriented on the right-hand side of the plot with the mesohaline salinity. But a high abundance of picoeukaryotes was found upstream. ANP was oriented on the left-hand side of the plot along with upstream locations, and their high abundance upstream was contributed by microcystis bloom. The RDA triplot showed that similar to the PRM, the smaller

plankton components in the KBW were high in the mesohaline conditions during the NEM also (Fig. 9c).

4. Discussion

The spatial hydrographical setting in the KBW is mainly controlled by the seasonal variations in the river discharge and tides (Jyothibabu et al., 2006; Haridevan et al., 2015; Anjusha et al., 2018). TB in the KBW has a significant role in regulating river discharge and tidal inflow (Haridevan et al., 2015; Arunpandi et al., 2020a,b; Arunpandi et al., 2021a,b), which in turn influences plankton functional groups (Haridevan et al., 2015; Anjusha et al., 2018; Arunpandi et al., 2020a,b). The smaller plankton community is a very sensitive and complex component and so their spatial distribution varies depending on hydrographical changes (Shurin et al., 2000; Cohen and Shurin, 2003; Cottenie et al., 2003; Leibold and Norberg, 2004) and their role in biogeochemical processes and carbon cycling in marine and freshwater environments are very significant (Cho and Azam, 1988; Arya et al., 2016). The advances in methods and simultaneous analysis of the ecosystem's physicochemical properties and biological components have facilitated investigations on the functional heterogeneity and ecology of smaller plankton in the aquatic environment (Postel et al., 2000; Seuront and Strutton, 2003).

The current study explained the possible response of the smaller plankton community to the altered hydrographical conditions in the downstream, midstream and upstream regions of the KBW caused by the establishment of the TB. As stated before, during the PRM, the closed TB restricts the saline intrusion to the midstream and prevents its spread upstream of the KBW (Arun, 2005, Haridevan et al., 2015; Anjusha et al., 2018; Arunpandi et al., 2020a,b). The spatial hydrographical setting in the KBW during the PRM was a polyhaline downstream, mesohaline midstream, and oligohaline/limnetic upstream (Arunpandi et al., 2020a) (Fig. 6a). Similar to the PRM, the river discharge is low during the NEM also (Qasim, 2003; Jyothibabu et al., 2006; Arunpandi et al., 2020a,b; Arunpandi et al., 2021a,b). As a result, TB shutters are partially opened during the NEM and at this time the spatial hydrographical setting in the KBW is mesohaline in the downstream, mesohaline to oligohaline in the midstream and oligohaline to limnetic in the upstream (Fig. 2b). During the SWM, the entire KBW is dominated by freshwater and hence there is no clear spatial segregation of salinity (Madhupratap, 1987; Menon et al., 2000; Qasim, 2003; Arun, 2005, Haridevan et al., 2015).

The DO and nutrient concentrations and their spatiotemporal variations in the KBW observed in the current study were very similar to the observations in many past studies (Saraladevi et al., 1983; Menon et al., 2000; Jyothibabu et al., 2006; Sooriya et al., 2015; Arya et al., 2016; Jagadeesan et al., 2017). During the SWM, turbidity was high throughout the whole KBW and low during the NEM and

PRM periods. Turbidity was 3 times higher during the SWM than during the PRM due to terrestrial inputs (Nasir, 2010) and was always high downstream. Seasonal and spatial variations in salinity significantly influence the biological components of the KBW (Madhupratap, 1987; Haridevan et al., 2015; Arunpandi et al., 2020a; Arunpandi et al., 2021c). The high nutrient concentration in KBW throughout the year sustains a high level of Chl-*a* biomass regardless of seasons contributed mainly by nanoplankton (Jyothibabu et al., 2006; Madhu et al., 2007; Sooriya et al., 2015; Jyothibabu et al., 2015).

Synechococcus and picoeukaryotes dominate the autotrophic picoplankton community in the KBW (Rajaneesh and Mitbavkar, 2013; Arya et al., 2016). It was observed earlier that the abundance of *Synechococcus* is usually higher in nutrient-rich waters (Partensky et al., 1996). The total APP abundance in the current study was found to be higher during the PRM as compared to the SWM (Fig. 6b). The slight differences in the salinity also make a significant effect on the growth and production of smaller plankton in the system (Rezayian et al., 2017; Kumar et al., 2021). During the PRM, *Synechococcus* (SYN I) abundance was higher in the polyhaline condition (Fig. 7a). The comparison of the locations on either side of TB showed higher APP in the midstream (north of TB) locations (Table 1). Regardless of the seasons, picoeukaryotes (PIE) numerically dominated over *Synechococcus* in the APP community. Their spatial distribution trend was just the inverse; picoeukaryotes were high in cluster IV in the upstream and low in the downstream, whereas *Synechococcus* was high in the downstream and low in the upstream. Studies have shown that different strains of picoplankton can exist in different hydrographical settings (Fuller et al., 2003; Arya et al., 2016). Studies have shown that different strains of picoplankton can exist in different hydrographical settings (Fuller et al., 2003; Arya et al., 2016). The Study by Rezayian et al., (2017) mentions that the salinity stress decreases the chlorophyll content and growth rate of smaller plankton and also pointed out SYN has limited adaptive potential to salinity. The changes in the salinity vary the content of phycoerythrin (PE- SYN I) and phycocyanin (PC- SYN II) in SYN (Rezayian et al., 2017).

In the current study, two strains of *Synechococcus* (SYN I and SYN II) were evident in the study area. The SYN I strain contributed 90% of the total *Synechococcus* abundance in cluster I locations downstream, but they decreased their abundance towards the upstream cluster IV locations. In cluster II locations in the midstream, the abundance of both strains (SYN I and SYN II) was found to be high and in equal proportions (Fig. 7a). On the other hand, SYN II was highly abundant in the cluster IV locations upstream and was significantly low in abundance in the cluster I location downstream. This was due to the difference in the salinity preference of these two strains of *Synechococcus*. SYN strains are capable to tolerate typically in moderate salinity conditions (Bemal and Anil, 2018). SYN I prefers

the euhaline/polyhaline condition whereas SYN II is the limnetic condition (Stomp et al., 2007; Wang et al., 2011; Arya et al., 2016). The studies also stated that the smaller plankton has some physiological adaptations to tolerate the changing salinity condition in the system (Brown, 1976; Qiu et al., 2012; Bernal and Anil, 2018; Kumar et al., 2021). During the SWM, the APP abundance was low in the entire stretch of the KBW compared to the PRM (Fig. 6d). At that time, oligohaline to limnetic conditions persisted in most parts of the KBW, which caused increased proportions of SYN II strains in the system. Also, the increased dilution of water by torrential rainfall and river discharge during the SWM may be instrumental in an overall decrease in plankton abundance (Jyothibabu et al., 2015).

During the NEM, the abundance of the total APP showed only small variations between locations, though the contribution of *Synechococcus* and picoeukaryotes varied spatially (Fig. 7c). The high abundance of APP was in cluster I downstream, where SYN I and SYN II strains were almost equal in proportion. SYN II and picoeukaryotes were found higher in cluster III upstream. Bernal and Anil, (2018) pointed out that the higher salinity affects the growth rate and duration of the cell-cycle stages of freshwater SYN stains. And it was noticed earlier that SYN I (PE-rich) was high in the coastal waters, while SYN II grows well in turbid freshwaters (Voros et al., 1998; Stomp et al., 2007; Wang et al., 2011). Studies also indicated that ANP was the major primary producer in the KBW, especially in the upstream (Jyothibabu et al., 2006; Madhu et al., 2007; Jyothibabu et al., 2015). However, during the PRM in the current study, ANP abundance was higher in cluster II midstream mesohaline locations, which was 2 to 3 folds higher compared to the downstream and upstream (Fig. 8b). The rapid freshwater discharge during the SWM considerably decreased the overall ANP abundance and, spatially, their higher abundance was found in the downstream locations compared to the upstream locations. ANP abundance was highest in cluster III upstream during the NEM, highest in cluster II midstream during the PRM, and highest in cluster I downstream and cluster II midstream during the SWM. During the NEM, a bloom of *Microcystis* (cyanobacteria) occurred upstream of the KBW. As a result, ANP increased overall. The growth, production, and carbon flow in the aquatic food web are all reduced when smaller plankton with lower salinity is advected into estuarine or marine habitats (Arya et al., 2016; Rezayian et al., 2017; Bernal and Anil, 2018; Kumar et al., 2021). And the changes in the cellular physiology of smaller plankton are a good indicator of their stress response to the environmental changes in the natural environment (Bernal and Anil, 2018).

NMDS plots and multivariate RDA plots highlight the ecological interrelationships between environmental and biological factors, demonstrating that mesohaline conditions are optimal for the highest abundance of pico and nanoplankton. This study clearly shows that salinity distributions in the KBW have a significant influence in determining the distribution pattern of several smaller plankton

components in the KBW. It would be interesting to learn how TB affects the natural distribution of plankton components by producing changes in salinity distribution via flow inhibitions. We compared the seasonal salinity distributions observed in the current study with a historical study before the creation of the TB to understand the trend in the long term change in the salinity distribution in the KBW and to infer its possible implications on the distribution pattern of smaller plankton components. Historical KBW data indicated that during the PRM, the downstream was euhaline, the midstream polyhaline, and the upstream mesohaline (Fig. 3). During the PRM, when the TB was closed in the current study, the downstream was polyhaline, the midstream was mesohaline, and the upstream was oligohaline (Table 1). Historically, the NEM was characterised by polyhaline downstream, mesohaline midstream, and oligohaline upstream (Haridas et al., 1973, Fig. 3), however in the current investigation, a polyhaline downstream, midstream, and limnetic upstream were found in the KBW (Fig. 6e). Both historical data and the current study demonstrated limnetic conditions across the KBW during the SWM, but more so during the former study period. Given that the mesohaline regions of the KBW support the greatest abundances of smaller autotrophic plankton communities, reducing salinity in the KBW may harm the smaller plankton components. The operation of TB has greatly reduced the mesohaline conditions in the upstream and midstream of KBW, reducing the geographical length of the optimal growing conditions for tiny plankton. TB has also produced seaward confinement of the optimum mesohaline growth conditions of smaller autotrophic plankton due to flow limitation. The mesohaline situation is the best since it benefits both groups. This demonstrates that TB, by modulating the salinity gradients in the KBW, significantly altered the distribution pattern of the smaller plankton components.

SWM is when the KBW receives the most freshwater, accounting for almost 70% of annual rainfall (Qasim, 2003; Jyothibabu et al., 2006; 2015; Arunpandi et al., 2021a, c). As a result, during SWM, the KBW was dominated by freshwater, whilst other seasons were dominated by saline water in downstream and upstream regions (Qasim, 2003; Arun, 2005; Jyothibabu et al., 2006). Globally, the saltwater barrage separates and stagnates water basins, causing a buildup of nutrients and contaminants in the system (Kumar, 2015). Furthermore, this saltwater barrage has a negative ecological influence on biological components, primarily plankton (Haridevan et al., 2015; Anjusha et al., 2018; Arunpandi et al., 2020a,b; Arunpandi et al., 2021a, b; Rashid et al.,). There are a few barrages in KBW to keep seawater out of the system, the largest of which is TB. This TB in the KBW has caused flow restrictions and water stagnation in the upstream region over the decades (Kannan, 1979; Gopalan et al., 1983; Balchand, 1983; WISA, 2013; Arunpandi et al., 2021 a,c), and it is worth noting that the salinity distribution in the KBW has decreased since the construction of the TB (Balchand, 1983, Arunpandi

et al., 2021 a,b). Furthermore, TB disrupts the normal ventilation and nutrient load via tidal activity in the KBW for an extended length of time (Gopalan et al., 1983; Balchand 1983; Dinesh Kumar, 1997, Arunpandi et al., 2021 a). TB affects planktonic food web components such as microzooplankton, mesozooplankton (cladocerans, copepods), and planktonic stages of black clam in the KBW by altering salinity levels and decreasing salinity in the upstream zone (Haridevan et al., 2015; Anjusha et al., 2018; Arunpandi et al., 2020a,b; Arunpandi et al., 2021a, b). Removal of TB is now impractical since it performs several critical functions in the region, including as preventing saline water from entering paddy fields in the upstream region and acting as a transit bridge between the regions (MSSRF, 2007; WISA, 2013; Kolathayar et al., 2021). A team of specialists have given a new proposal in 2011, which was to keep the TB shutters open every day for a specific length of time to let saline water into the upstream region to minimise the accumulation of nutrients and pollution (WISA, 2013). The amount of salinity rise in the upstream region, on the other hand, should be clearly defined and regulated based on a sound scientific basis.

5. Conclusion

The current research describes the distribution of tiny plankton (pico- and nano-plankton) in the KBW and shows how substantial hydrographic changes generated by a huge saltwater barrage (TB) altered their distribution pattern. Mesohaline conditions in the KBW were found to be optimal for increasing the abundance of both pico and nanoplankton communities. A comparison of the existing seasonal salinity distribution with that before the installation of the TB revealed a considerable decrease in the KBW's current salinity levels. The current study illustrated how the changing salinity distribution caused by TB flow restrictions might impact the distribution of smaller plankton components across seasons. The current research found two *Synechococcus* strains (SYN I and SYN II), which were identified in similar numbers in the mesohaline zones. The overall results of this study showed that TB in the KBW reduced the geographical extent and spatial orientation of the mesohaline region in the upstream and midstream of the system thereby adversely affecting the natural abundance and distribution pattern of smaller plankton. Hence, to facilitate more mesohaline salinity levels in the KBW, a more frequent opening of the shutters of the TB based on a sound ecological understanding has been suggested for the better management of the biological resources in this large Ramsar wetland system in Asia.

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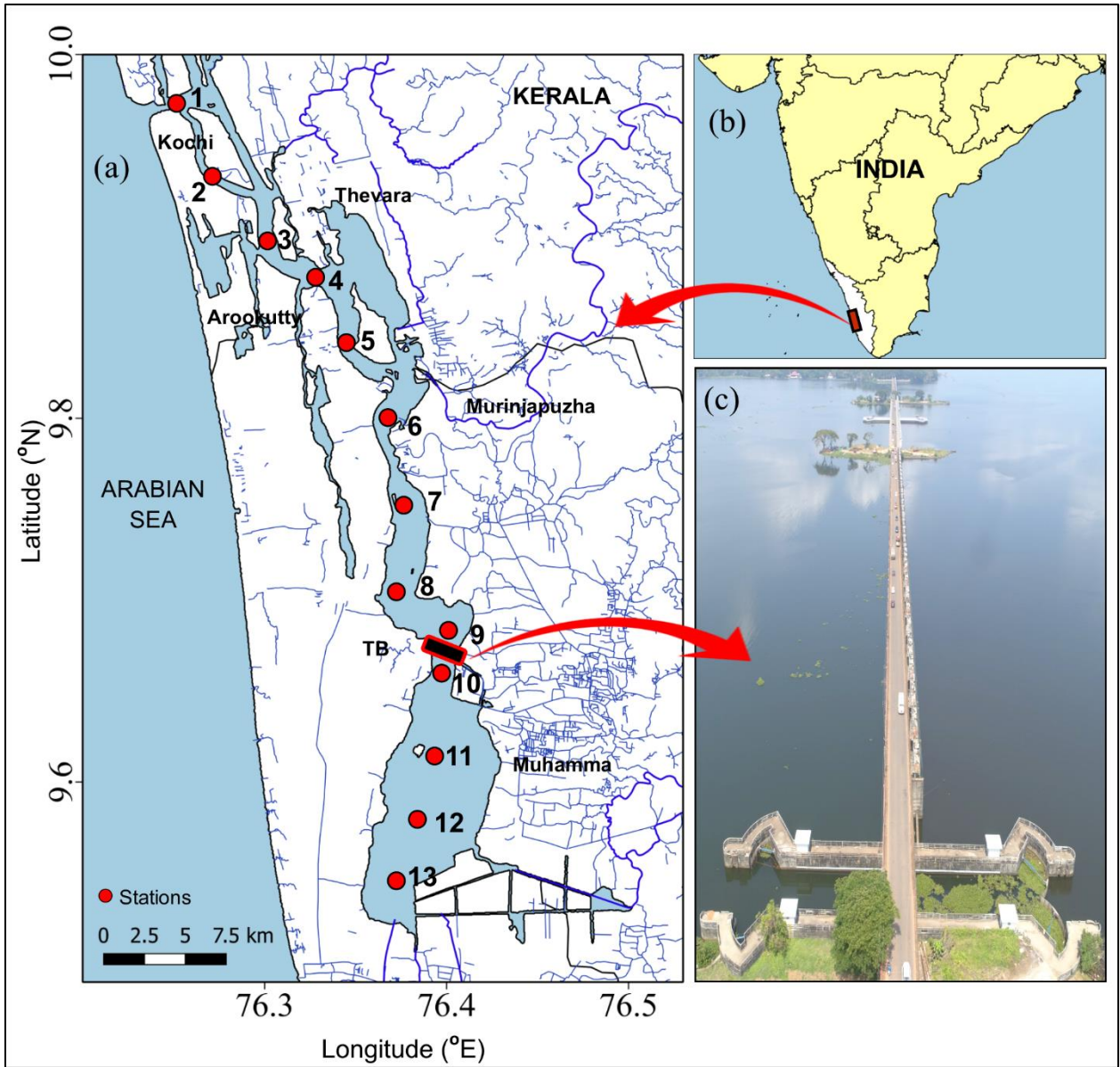


Fig. 1 - Sampling locations in the Kochi Backwaters. TB - Thannermukkom barrage.

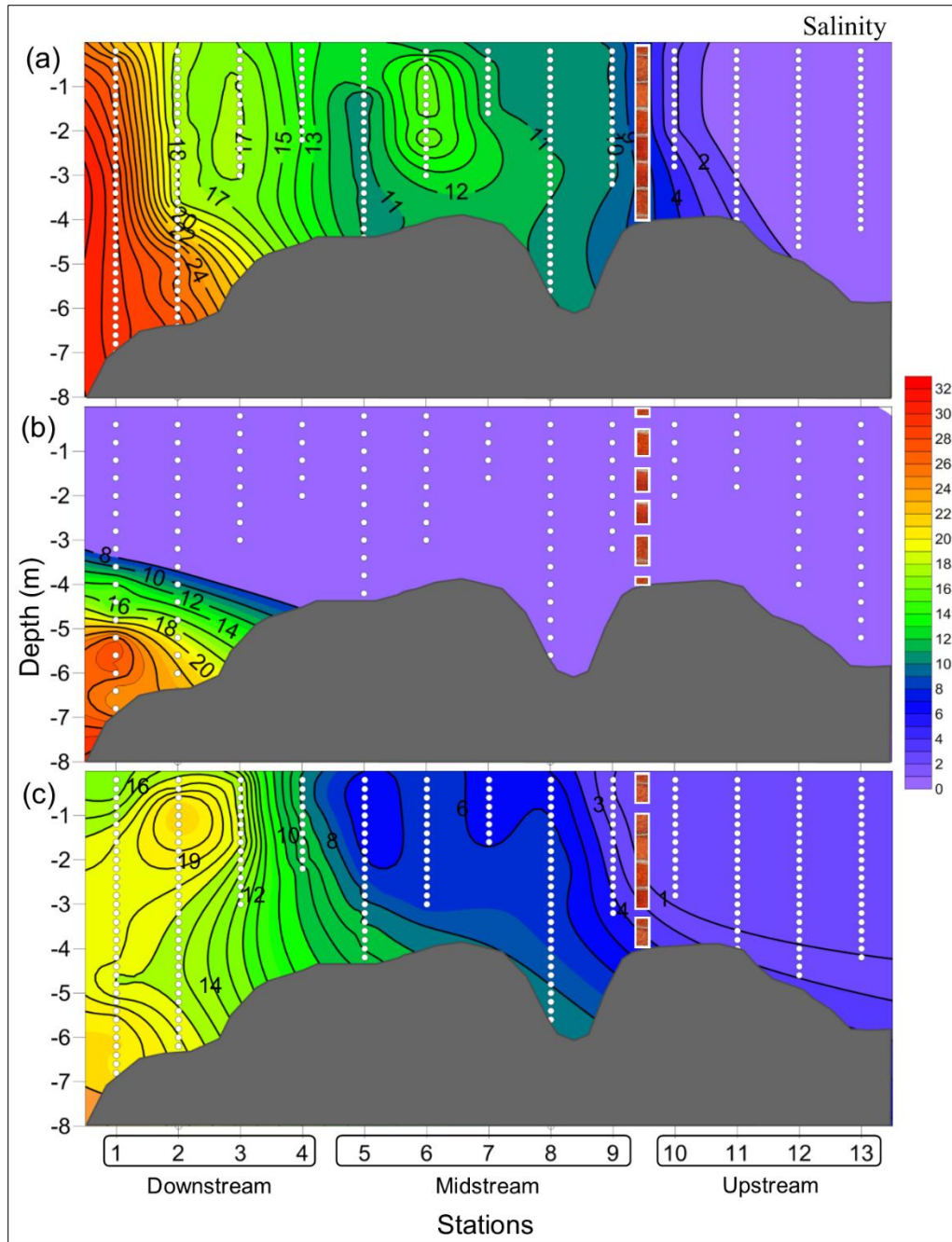


Fig. 2 - The vertical distribution of salinity in the Kochi backwaters. CTD data points are represented as white dots, and the thick red discontinuous line indicates the Thannermukam barrage (TB) across the KBW. (a) Pre-Monsoon [(PRM); TB shutters fully closed], (b) Southwest Monsoon [(SWM); TB shutters fully open] and (c) Northeast Monsoon [(NEM) - TB shutters partially open].

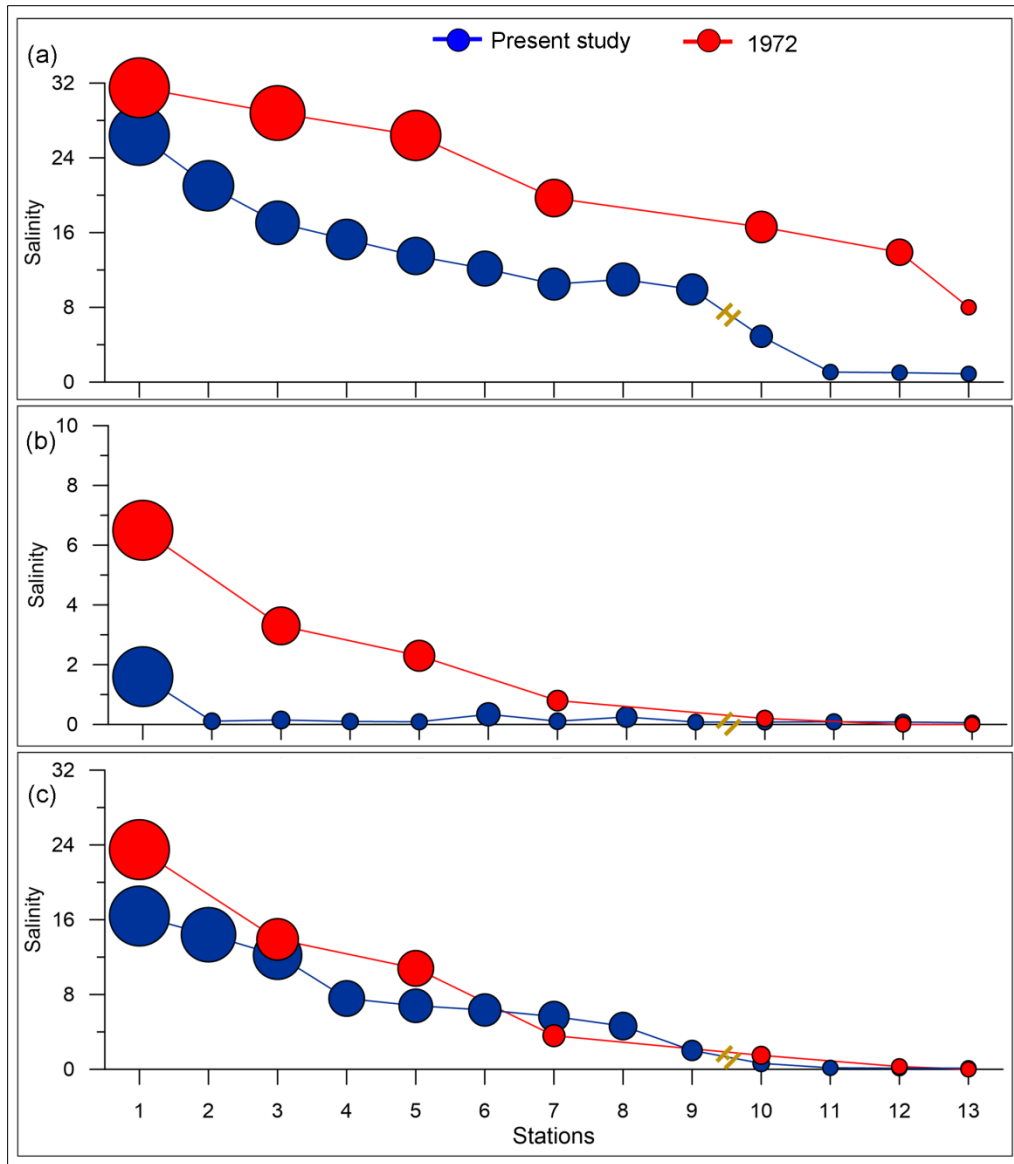


Fig. 3 - Comparison of the present salinity (blue line and bubbles) with the historical data points (red line and bubbles) before TB was operational in the KBW. (a) Pre-Monsoon (PRM), (b) Southwest Monsoon (SWM) and (c) Northeast Monsoon (NEM).

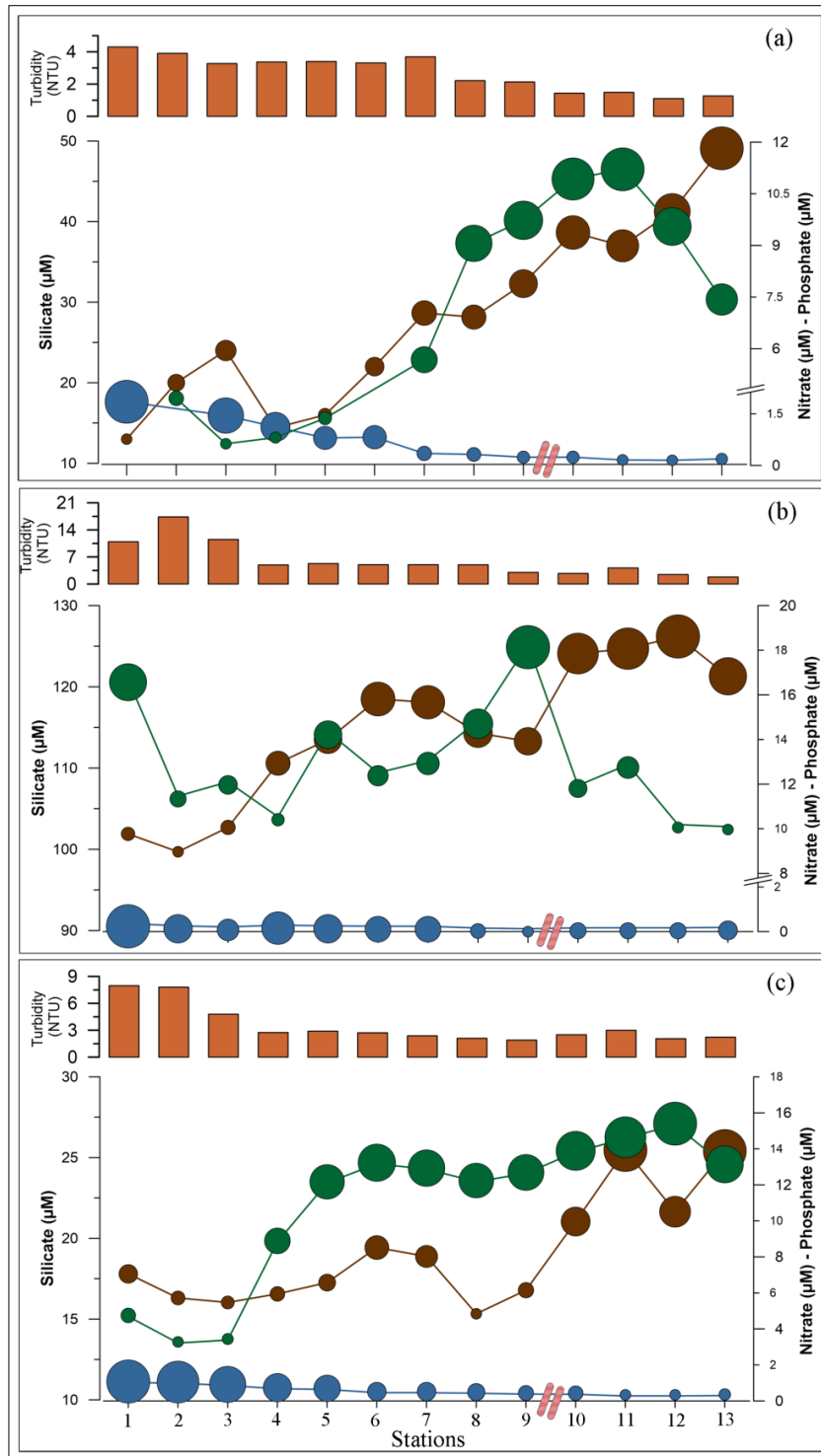


Fig. 4 - Distribution of macronutrients [silicate (brown line and bubbles), nitrate (green line and bubbles), and phosphate (blue line and bubbles),] in the KBW during (a) Pre-Monsoon (PRM), (b) Southwest Monsoon (SWM) and (c) Northeast Monsoon (NEM). Vertical bars indicate turbidity level.

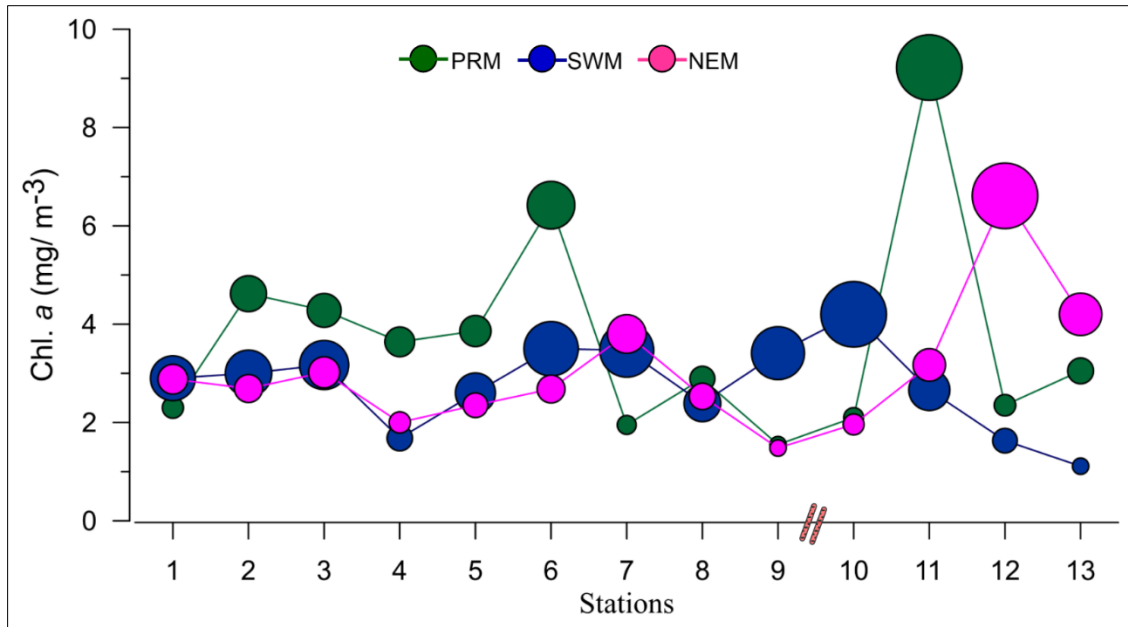


Fig. 5 - Distribution of chlorophyll *a* in the KBW during Pre-Monsoon (PRM), Southwest Monsoon (SWM) and Northeast Monsoon (NEM).

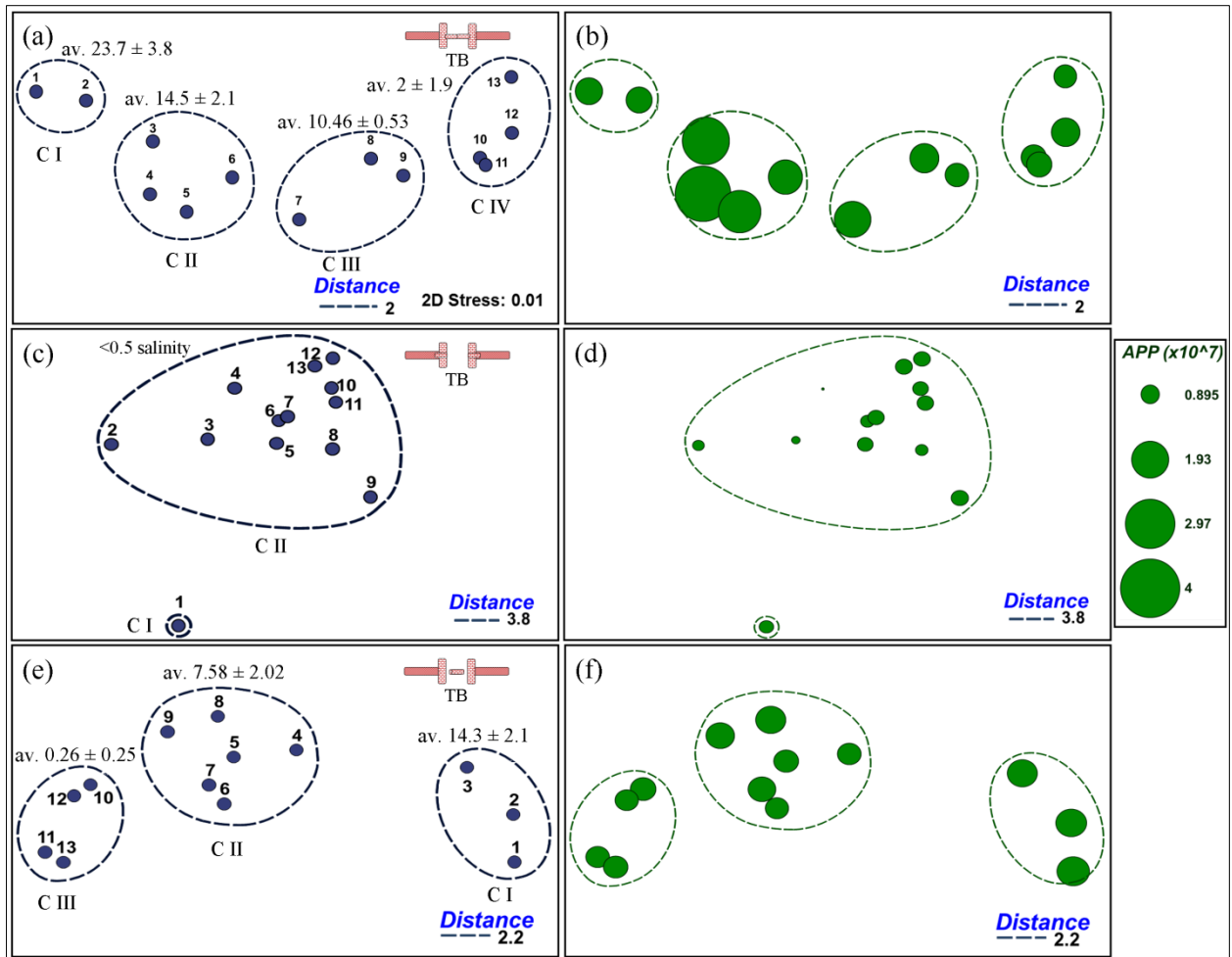


Fig. 6 - Cluster and NMDS plots presenting the separation of locations based on the distribution of physicochemical variables in the KBW during (a) Pre-Monsoon (PRM), (b) Southwest Monsoon (SWM) and (c) Northeast Monsoon (NEM). Subsequent panels show physicochemical NMDS plots overlaid with bubbles of autotrophic picoplankton [APP ($\times 10^7$) cells/L] during (b) PRM, (d) SWM and (f) NEM. Average salinity values are presented above each cluster and the functional state of the Thaneermukkam barrage (TB) is represented in red colour.

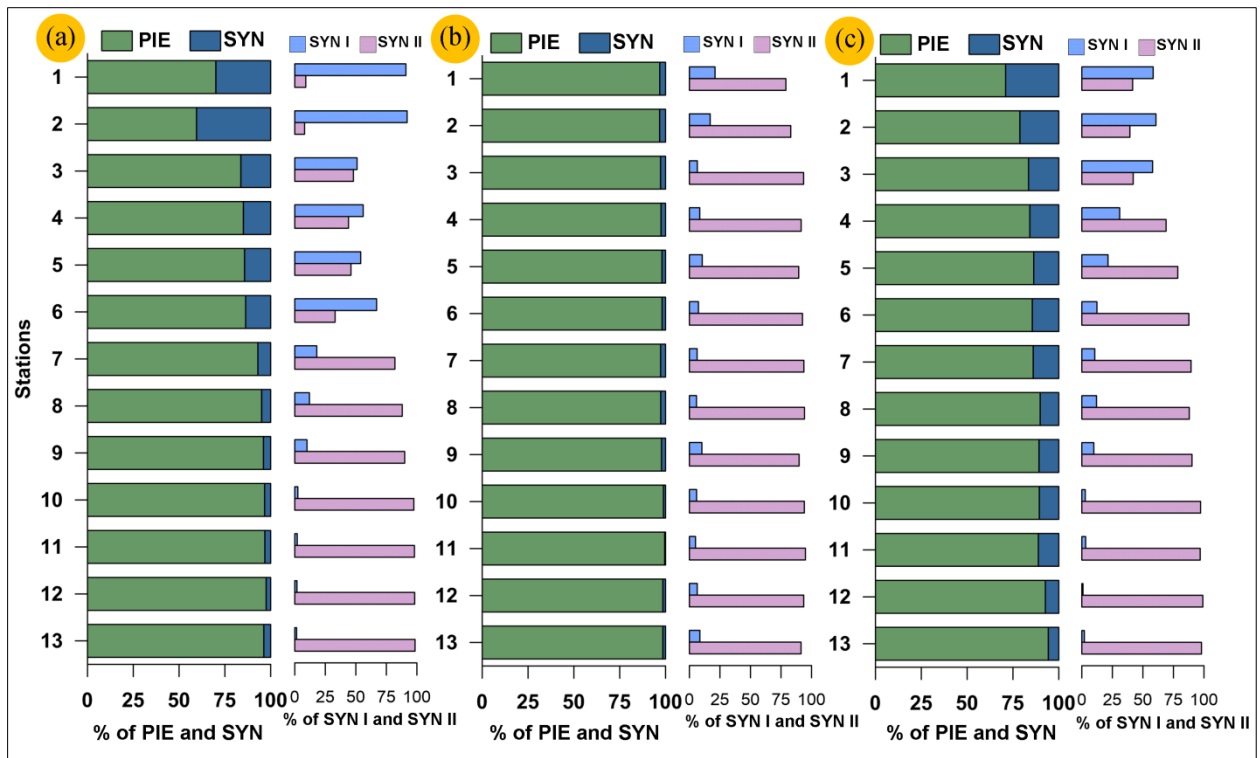


Fig. 7 - Percentage contribution of *Synechococcus* (SYN) and picoeukaryotes (PIE) in the KBW during (a) Pre-Monsoon (PRM), (b) Southwest Monsoon (SWM) and (c) Northeast Monsoon (NEM). The two strains of *Synechococcus* (SYN I) strain in blue, and the phycocyanin-rich (SYN II) strain in pink.

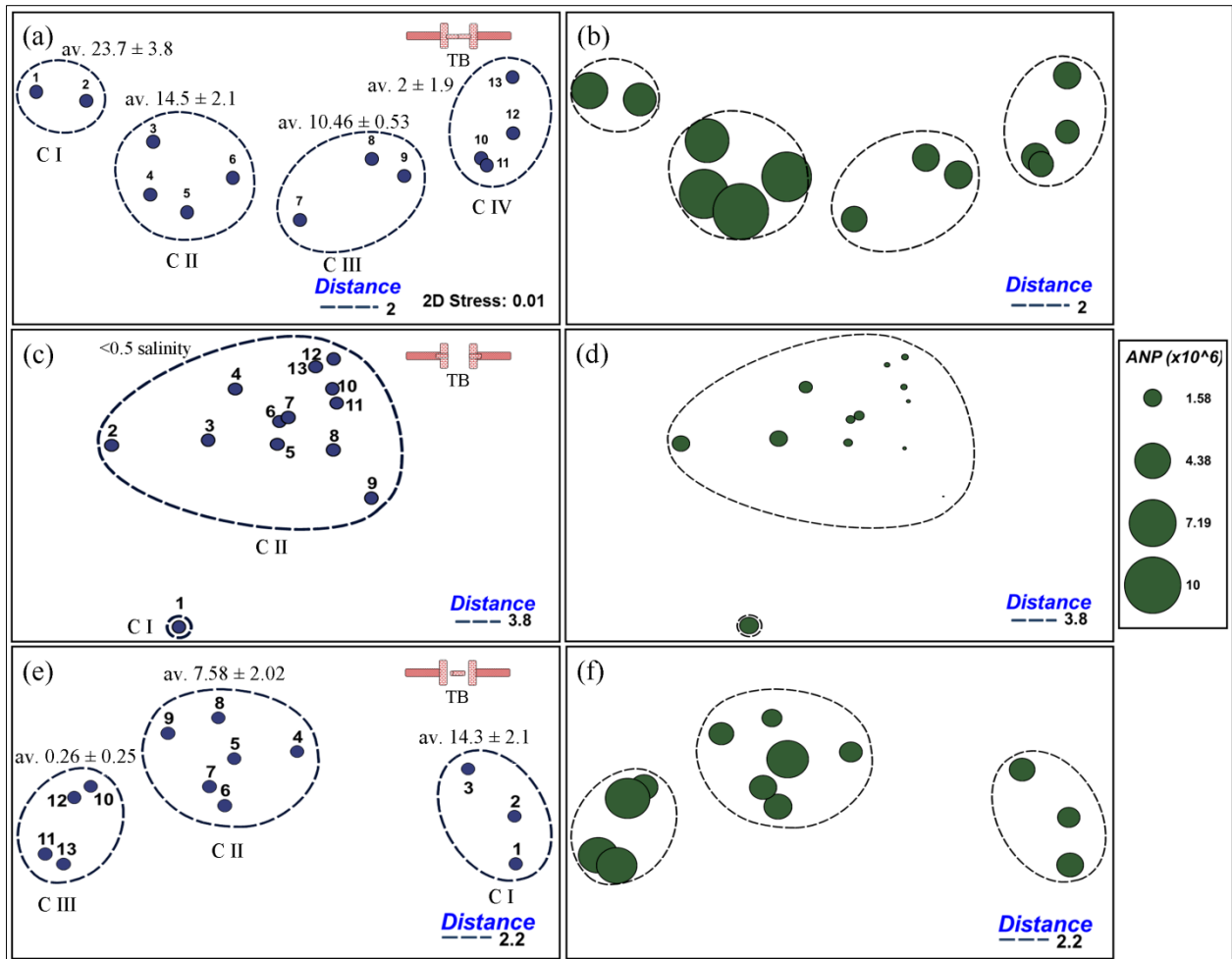


Fig. 8 - Cluster and NMDS plots present the separation of locations based on the distribution of physicochemical variables in the KBW during (a) Pre-Monsoon (PRM), (b) Southwest Monsoon (SWM) and (c) Northeast Monsoon (NEM). Subsequent panels show physicochemical NMDS plots overlaid with bubbles of autotrophic nanoplankton [ANP ($\times 10^6$) cells/L] during (b) PRM, (d) SWM and (f) NEM. Average salinity values are presented above each cluster and the functional state of the Thaneermukkam barrage (TB) is represented in red colour.

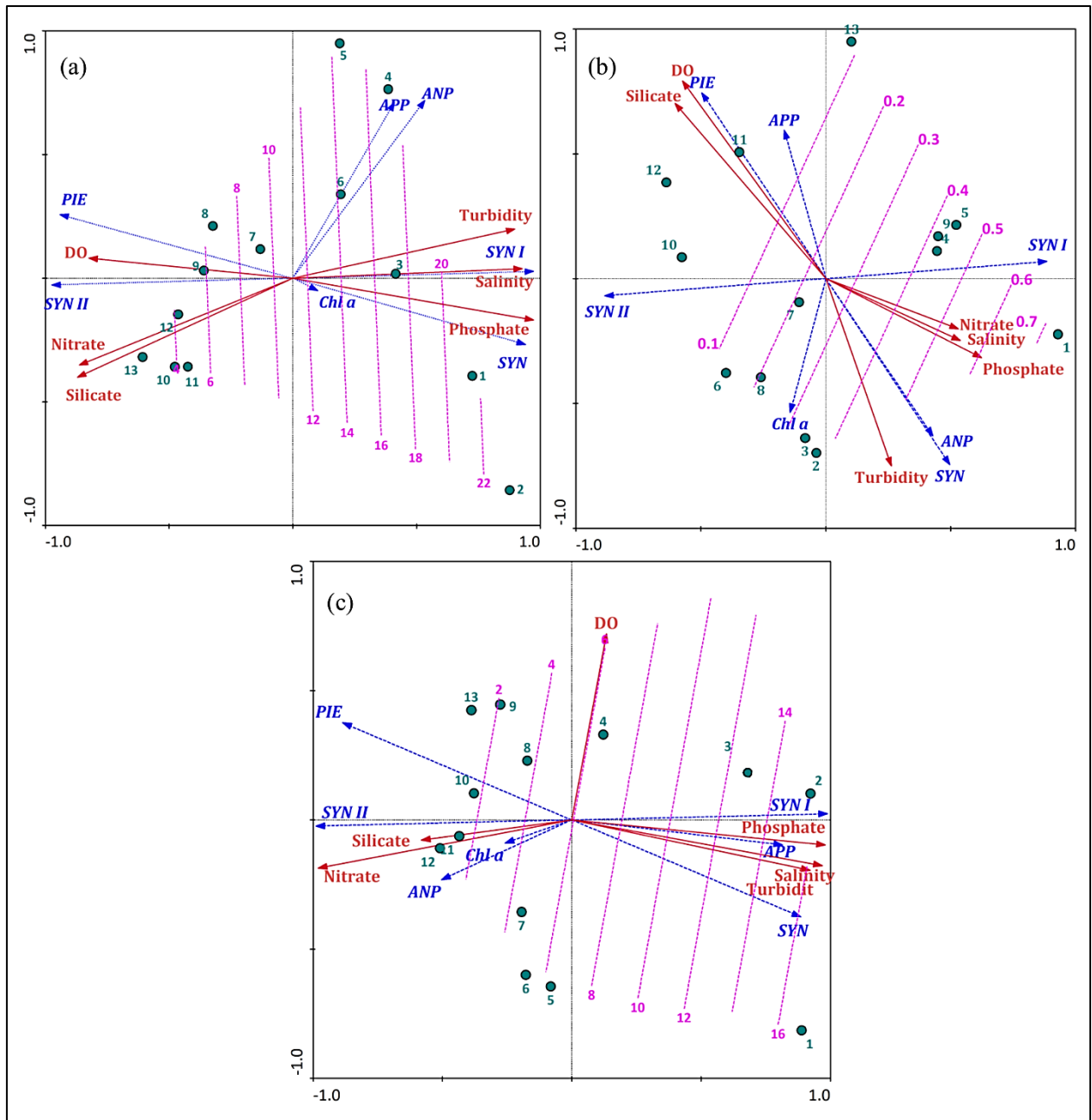
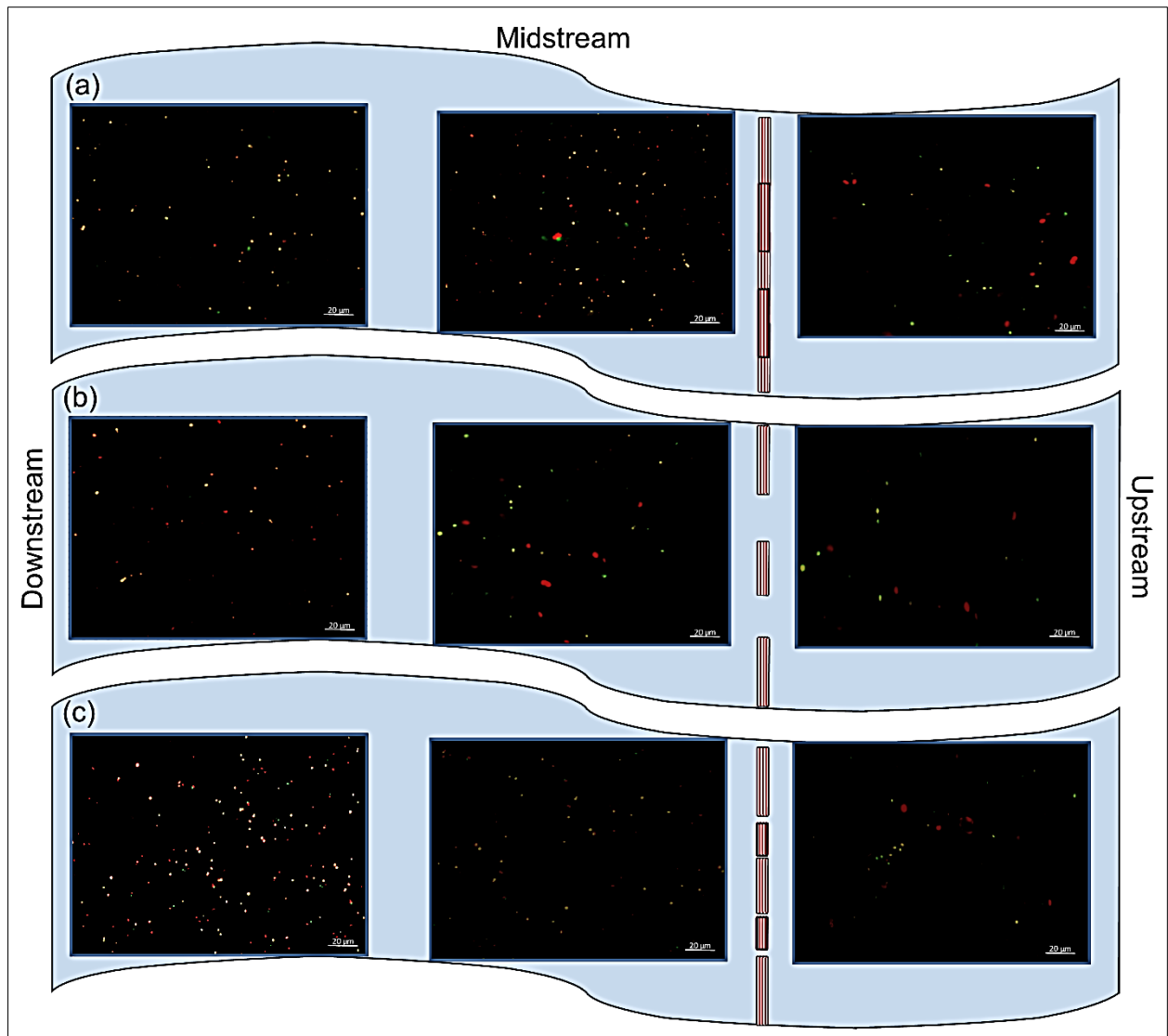
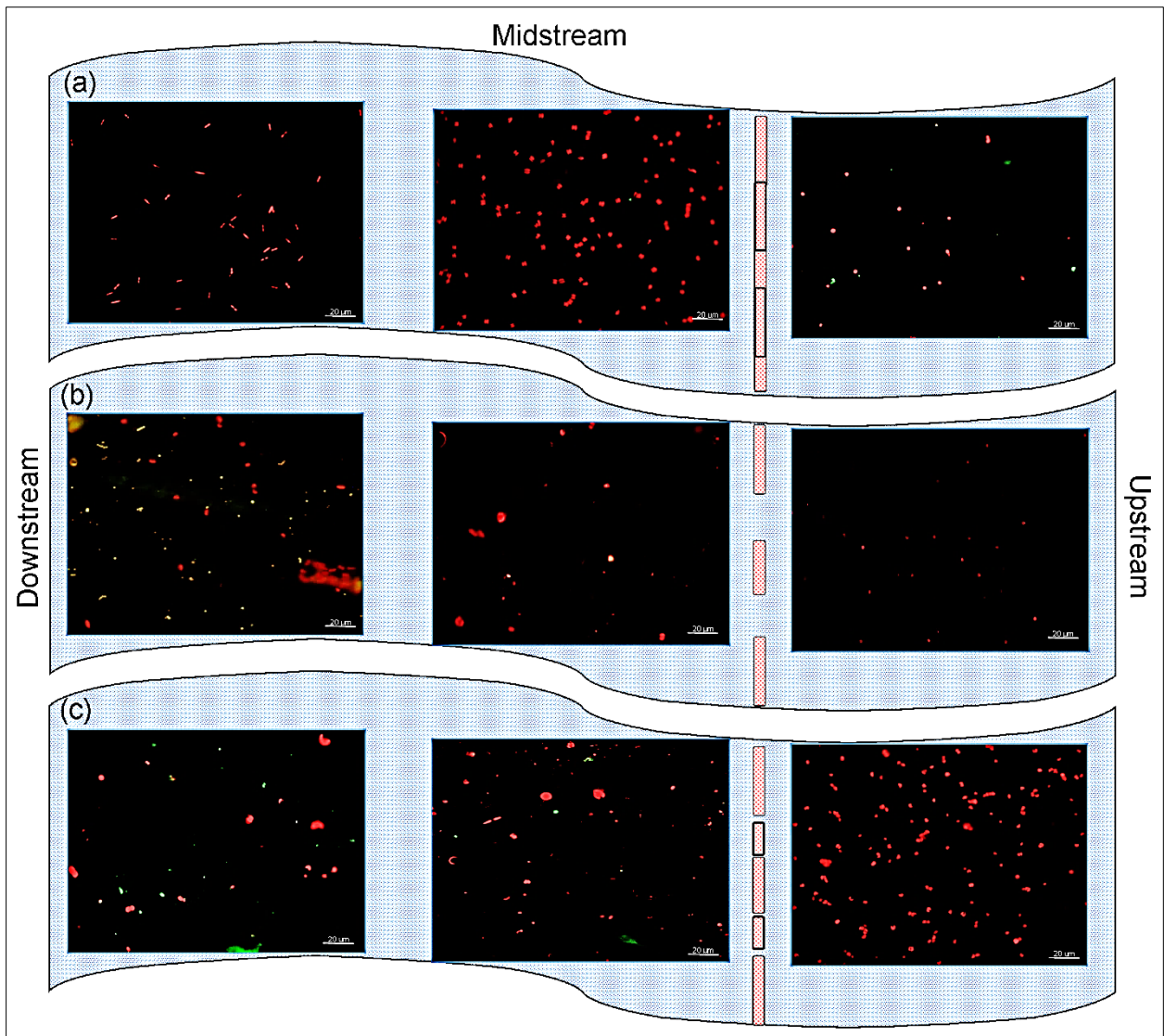


Fig. 9 - RDA triplot displaying the distribution and multiple correlations of physicochemical and biological variables during (a) Pre-Monsoon (PRM), (b) Southwest Monsoon (SWM) and (c) Northeast Monsoon (NEM). The overlaid pink dotted line attribution contours represent the spatial distribution of salinity and its interrelationship with other physicochemical and biological variables. Biological and environmental variables are presented as arrows; the blue-dotted arrows indicate biological components, and the red arrows environmental variables. Abbreviations: Chl *a*- Chlorophyll-*a*; DO - Dissolved Oxygen; SYN- *Synechococcus*; PIE- Picoeukaryotes; ANP- Autotrophic nanoplankton.



Supplemental material 1 - Photomicrographs of autotrophic picoplankton (APP) in the KBW during (a) Pre-Monsoon (PRM), (b) Southwest Monsoon (SWM) and (c) Northeast Monsoon (NEM). Two strains of *Synechococcus* are represented in the panels: phycoerythrin rich (SYN I) strain is yellow/orange, and phycocyanin-rich (SYN II) strain is red.



Supplemental material 2 - The photomicrographs of autotrophic nanoplankton (ANP) in the KBW during (a) Pre-Monsoon (PRM), (b) Southwest Monsoon (SWM) and (c) Northeast Monsoon (NEM).

Table 1 - Spatial and seasonal distribution of environmental and biological (surface only) variables in KBW

		Stations												
Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	
PRM	Salinity	26.4	21.01	17.05	15.27	13.5	12.15	10.49	10.99	9.92	4.9	1.07	1.01	0.89
	DO (mg/L)	3.98	4.47	4.95	4.76	4.92	5.31	5.63	5.13	5.74	5.24	5.06	6.00	5.92
	NO ₃ (µM)	2.81	1.95	0.63	0.81	1.37	3.46	5.68	9.06	9.73	10.93	11.21	9.55	7.43
	PO ₄ (µM)	1.85	2.05	1.45	1.12	0.80	0.82	0.35	0.32	0.24	0.24	0.16	0.15	0.19
	Sio ₄ (µM)	13.00	20.00	24.00	14.40	16.0	22.00	28.65	28.15	32.30	38.65	37.00	41.25	49.10
	Turbidity(NTU)	4.30	3.90	3.27	3.37	3.40	3.31	3.69	2.21	2.13	1.43	1.48	1.10	1.26
	Chl <i>a</i> (mg/ m ³)	2.3	4.62	4.28	3.64	3.86	6.42	1.95	2.89	1.55	2.1	9.22	2.35	3.05
	APP (x10 ⁷) Cells/L	1.37	1.31	3.05	4.01	2.60	1.87	2.03	1.52	1.17	1.25	1.26	1.47	1.14
	PIE (%)	70.1	59.5	83.8	85.1	85.7	86.3	93	95.1	96.1	96.7	96.8	97.6	96.3
	SYN (%)	29.9	40.5	16.2	14.9	14.3	13.7	7	4.9	3.9	3.3	3.2	2.4	3.7
	ANP (x10 ⁶) Cells/L	4.60	3.90	6.44	8.00	10.08	8.23	2.64	2.89	2.97	3.02	2.54	2.35	2.88
SWM	Salinity	1.60	0.11	0.15	0.10	0.09	0.34	0.11	0.25	0.08	0.08	0.09	0.08	0.06
	DO (mg/L)	5.78	5.91	5.95	6.01	6.09	6.17	6.2	6.2	6.26	6.7	6.98	7.01	7.06
	NO ₃ (µM)	16.96	11.73	12.36	10.80	14.61	12.77	13.32	15.10	18.53	12.20	13.14	10.45	10.36
	PO ₄ (µM)	0.37	0.26	0.21	0.29	0.26	0.24	0.24	0.16	0.13	0.17	0.17	0.17	0.19
	Sio ₄ (µM)	101.9	98.7	102.7	110.6	113.5	118.5	118.1	114.3	113.3	124.1	124.7	126.2	121.3
	Turbidity(NTU)	10.92	17.37	11.52	4.94	5.28	4.99	4.99	4.98	3.02	2.73	4.15	2.45	1.83
	Chl <i>a</i> (mg/ m ³)	2.9	3	3.17	1.68	2.6	3.5	3.47	2.39	3.41	4.2	2.66	1.63	1.11
	APP (x10 ⁷) Cells/L	0.80	0.72	0.64	0.56	0.85	0.78	0.88	0.74	0.92	0.86	0.88	0.89	0.91
	PIE (%)	96.9	96.8	97.3	97.6	98.1	98.1	97.3	97.4	97.8	98.8	99.5	98.6	98.6
	SYN (%)	3.1	3.2	2.7	2.4	1.9	1.9	2.7	2.6	2.2	1.2	0.5	1.4	1.4
	ANP (x10 ⁶) Cells/L	1.83	1.69	1.70	1.22	0.88	0.89	1.00	0.69	0.65	0.78	0.69	0.79	0.74
NEM	Salinity	16.4	14.4	12.2	7.58	6.80	6.35	5.65	4.63	2.02	0.64	0.16	0.12	0.12
	DO (mg/L)	4.05	5.09	5.08	4.8	4.09	4.05	4.16	4.85	4.47	4.87	4.92	4.72	4.36
	NO ₃ (µM)	4.57	3.1	3.26	8.71	11.99	13.05	12.75	12.06	12.51	13.71	14.46	15.22	12.96
	PO ₄ (µM)	1.02	0.99	0.86	0.68	0.64	0.46	0.46	0.43	0.38	0.37	0.28	0.28	0.30
	Sio ₄ (µM)	17.80	16.31	16.03	16.56	17.26	19.43	18.88	15.33	16.79	21.04	25.47	21.65	25.41
	Turbidity(NTU)	7.97	7.80	4.79	2.75	2.90	2.72	2.38	2.10	1.90	2.50	2.99	2.06	2.23
	Chl <i>a</i> (mg/ m ³)	2.88	2.69	3.02	2	2.35	2.68	3.8	2.53	1.48	1.96	3.17	6.61	4.2
	APP (x10 ⁷) Cells/L	2.12	1.81	1.58	1.20	1.25	1.17	1.41	1.59	1.49	1.28	1.19	1.16	1.23
	PIE (%)	71	78.8	83.5	84.2	86.3	85.6	86.1	89.9	89.3	89.4	88.9	92.7	94.3
	SYN (%)	29	21.2	16.5	15.8	13.7	14.4	13.9	10.1	10.7	10.6	11.1	7.3	5.7
	ANP (x10 ⁶) Cells/L	2.91	2.18	2.70	2.22	5.96	3.08	2.88	1.84	2.56	3.06	5.54	6.82	5.65

Table 2 - Statistical Comparison (Kruskal- Wallis ANOVA -Non parametric ANOVA) of the hydrographical and biological parameters between the clusters during Pre-Monsoon [(PRM), Southwest Monsoon (SWM) and Northeast Monsoon (NEM). The symbol * denotes statistically significance $p < 0.05$. NS – Non-significant.

Parameters	PRM		SWM		NEM	
	H (Chi ²)	P value	H (Chi ²)	P value	H (Chi ²)	P value
Salinity	11.18	0.011*	2.28	0.134 NS	10.41	0.005*
DO	7.87	0.048*	1.19	0.17 NS	2.29	0.32 NS
NO ₃	9.54	0.021*	0.16	0.693 NS	9.75	0.007*
PO ₄	10.98	0.014*	3.56	0.06 NS	10.44	0.005*
Sio ₄	10.58	0.018*	4.71	0.03*	8.01	0.018*
Turbidity	9.95	0.019*	3.98	0.048*	6.42	0.048*
Chl <i>a</i>	8.14	0.041*	0.04	0.84 NS	3.11	0.21 NS
APP	10.43	0.015*	0.97	0.32 NS	6.73	0.034*
PIE	9.58	0.024*	0.96	0.32 NS	6.75	0.034*
SYN I	11.48	0.010*	4.71	0.03*	10.38	0.005*
SYN - II	8.91	0.03*	3.89	0.048*	6.09	0.047*
ANP	9.94	0.019*	3.89	0.048*	4.85	0.088 NS