

Microplastics in copepods reflects the manmade flow restrictions in the Kochi backwaters, along the Southwest coast of India

**Rashid, C.P., Jyothibabu, R^{*}, Arunpandi, N., Santhikrishnan, S., Vidhya, V., Sarath, S.,
Arundhathy, M., Alok, K.T**

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

^{*}Corresponding author. ^{*}E-mail rjyothibabu@nio.org

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

Abstract

This baseline study on microplastics (MPs) in calanoid copepods in the Kochi backwaters (KBW), India's largest estuary system on the west coast, focuses on (a) the spatiotemporal variations of MPs with the seasonal hydrography setting, and (b) how man-made flow restrictions of a large saltwater barrage contribute to MPs in copepods and their potential to transfer to higher trophic levels. This study found that MPs in copepods in the KBW ranged from av. 0.01 ± 0.014 to 0.11 ± 0.03 no./ind. seasonally. When the saltwater barrage shutters were fully/partially closed during the Pre-monsoon/Northeast Monsoon, MPs in copepods were considerably larger (av. 0.11 ± 0.03 no./ind., and av. 0.075 ± 0.02 no./ind., respectively) as compared to the Southwest Monsoon (av. 0.03 ± 0.01 no./ind.), when the barrage shutters were fully open. This shows the potential of man-made flow restrictions to increase the bioconcentration of MPs in copepods and their possible transfer to higher trophic levels through the food chain, adding to the region's previous discovery that much higher trophic level resources are polluted with a high concentration of MPs.

Keywords: mesozooplankton, copepods, microplastics, barrage, Kochi backwaters

1. Introduction

In recent decades, MPs have become a major environmental contaminant as their small size (<5 mm) allows them to be bioavailable and cause harmful effects on a wide range of aquatic biota (Cole et al., 2013, Avio et al., 2016, Rochman et al., 2019; Karuppasamy et al., 2020; Nikki et al., 2021; Rashid et al., 2021). Globally, it is estimated that 1.2-2.4 million tonnes of plastic garbage are carried by rivers and disposed of into the sea each year (Lebreton et al., 2017; Su et al., 2019; Xu et al., 2020). MPs ingestion has been reported on a wide range of marine organisms, including amphipods, barnacles (Thompson et al., 2004) mussels (Browne et al., 2008; Naidu, 2019) decapod crustaceans (Murray et al., 2011) and fishes (Hossain et al., 2019; Thiele et al., 2021). Consumption of microplastics can result in adverse health impacts, including reduced feeding, loss of energy reserves, obstructing feeding appendages, blocking the alimentary tract, reduced fecundity and survival (Cole et al., 2015; Gregory, 2009). Further, MPs may act as a vector that introduces toxicants to the organisms: additives and water-borne chemicals. Uptake of contaminated MPs by aquatic organisms provides a possible way for the introduction of these hazardous substances into the aquatic food web (Thompson, 2015; Andrady and Zhu, 2021). The majority of them originate from land-based sources, and estuaries act as a crucial pathway for their transfer to the oceans (Arthur et al., 2009; Wang et al., 2017; Su et al., 2019; Xu et al., 2020).

The Kochi Backwaters (KBW) is India's second-largest estuarine system (256 km²), and seven rivers contribute their influx into it, resulting in a massive amount of freshwater reaching there, especially during the southwest monsoon (June-September), when the region receives more than 70% of its annual rainfall (Qasim, 2003; Jyothibabu et al., 2006; Haridevan et al., 2015; Arunpandi et al., 2020; 2021a). Thus, the KBW behaves as a freshwater dominant system during the SWM, but during the dry Pre-Southwest Monsoon (PSWM; March to May) and Northeast Monsoon (NEM-November to February), it behaves as a saltwater dominant system (Qasim, 2003; Jyothibabu et al., 2006; Kurup, 1990; Srinivas et al., 2003). In 1976, a saltwater barrage was constructed at Thanneermukkom upstream of the KBW to prevent saline water intrusion into agricultural fields further upstream in the Kuttanad region during the non-monsoon season (Jyothibabu et al., 2006; Arunpandi et al., 2020; 2021b). The hydraulic shutters of the Thanneermukkom Barrage (TB) stay entirely open during the SWM to remove floodwater from the upstream zone of KBW into the neighbouring Arabian Sea. However, TB stays partially

closed during the NEM and completely closed during the PSWM to prevent saline water intrusion into the Kuttanad region (Jyothibabu et al., 2006; Arunpandi et al., 2020; 2021a & b). During this time, TB separates the KBW partially or completely into two sections: the brackish water downstream and the virtually freshwater (limnetic) upstream (Haridevan et al., 2015; Anjusha et al., 2018; Arunpandi et al., 2020). Because of these flow restrictions imposed by TB, the natural environmental settings of KBW has significantly altered impacting the biological components, especially during the PSWM and NEM periods (Arunpandi et al., 2020; 2021b). During this time, the isolated upstream of KBW becomes a reservoir for many pollutants, which adversely affects many biological resources inhabited there (Ouseph, 1992; Balachandran et al., 2003; Kaladharan et al., 2011; Anu et al., 2014; George et al., 2016; Gandhi et al., 2017, Arunpandi et al., 2020; 2021b). Hence, TB is commonly referred to as an 'ecological backlash,' in which its adverse effects outweigh the intended effects (Gopalan et al., 1983; Balchand et al., 1983).

Zooplankton (200-20000 μ m) play an important role in the aquatic food chain by linking primary producers and higher trophic level animals (Jyothibabu et al., 2006; Jagadeesan et al., 2013; 2017; Arunpandi et al., 2017; 2020). In general, copepods account for more than 70% of the mesozooplankton community in the Indian seas and estuarine systems (Jagadeesan et al., 2013; 2017; Jyothibabu et al., 2018; Arunpandi et al., 2017; 2021a). As copepods freely move along with the water currents, they are considered very good indicators of changes in environmental variables (Jagadeesan et al., 2013; Jagadeesan et al., 2017). Many earlier studies have been there on MPs contamination in KBW, and their salient observations have been summarised in Table 1. These studies have either addressed water, sediment, or species of the higher trophic levels (Kripa et al., 2012; Sruthy and Ramasamy, 2017; Devi et al., 2020; Suresh et al., 2020; Nikki et al., 2021). The bioavailability of MPs to copepods depends on several biotic and abiotic factors including abundance, size, shape, colour, chemical composition and ageing of MPs (Rashid et al., 2021). Most MPs have the same size as copepods usual food items such as pico and nanoplankton, making them consumable by copepods during their filter-feeding activity (Botterell et al., 2019; Rashid et al., 2021). MPs concentrated in copepods have the potential to transfer to higher trophic levels through the food chain (Farrell and Nelson, 2013; Setala et al., 2014; Botterell et al., 2019; Rodrigues et al., 2021). However, the MPs in zooplankton of Indian estuaries have never been quantified, and our recent study in the eastern

Arabian Sea is the first of its kind from the whole Indian subcontinent's nearshore waters (Rashid et al., 2021). Considering all of these, it is hypothesised that alteration of the natural hydrographical settings by TB leaves obvious imprints on the current ecological settings and that the copepods in the KBW may have MPs intake that varies with the flow restrictions. Hence, this baseline investigation on MPs in copepods in the KBW based on seasonal sampling measures (a) MPs concentration in copepods in 3 hydrographical zones of the KBW during the 3 different seasons and (b) how manmade flow constraints of TB affect MPs concentration in copepods in the KBW.

2. Materials and Methods

2.1. Study area and sampling

The KBW is India's second-largest estuarine system and is located in the state of Kerala on India's southwest coast between 9° 40' and 10° 12' N and 76° 10' and 76° 30' E (Figure 1). Seasonal sampling was carried out in August 2017 (SWM), December 2017 (NEM) and March 2018 (PSWM). Six locations were sampled during all three seasons, which represented different sections of the KBW; locations 1 and 2 in the downstream, 3 and 4 in the middle, and 5 and 6 in the upstream. To demarcate the difference in hydrographical settings in different sections of the KBW, the necessary hydrographical parameters were measured using standard procedures. The sampling was conducted in a speed boat, and the vertical profiles of temperature and salinity were measured using a conductivity, temperature, and depth (CTD) profiler (Seabird). Turbidity was measured using a turbidity meter (Eutech TN-100). A working party zooplankton net (Hydrobios, 0.5 m diameter, 200 µm mesh) was utilised to collect mesozooplankton samples from just below (1 m below) the water surface. A flow metre (Hydrobios) was used to determine the volume of water filtered through the net during the sampling. The net was operated with utmost care and it was thoroughly cleaned before and after each sampling. Zooplankton samples were transferred to a 500 ml pre-cleaned container and stored in 4% formalin. In the laboratory, as a preliminary procedure, all debris in the samples was removed and zooplankton was separated into two halves using a Folsom plankton splitter. One half of the zooplankton was sorted up to group level, while the other half was utilised for MPs analysis. Copepods were recognized up to the species level using standard literature (Tanaka, 1956; Kasturirangan, 1963; Sewell, 1999; Conway et al., 2003).

2.2 MPs analysis in zooplankton

Only copepods of the order Calanoida were examined for MPs analysis in this study, as they are the dominant ones in the KBW and also because the individuals of the other two orders of copepods (Cyclopoida and Harpacticoida) have significantly lower size ranges (av. 0.72 ± 0.12 mm) that fit within the size range of the MPs and are thus less likely to ingest them. 100 calanoid copepods larger than 500 μm was manually picked from the zooplankton samples collected from each site. Milli Q water was used to clean each copepod specimen several times. Under a stereo zoom microscope (Zeiss Stemi 305), each of these specimens was thoroughly cross-checked to any adhered debris, including MPs, to their bodies. All adhered debris was removed with pointed forceps under the microscope. The specimens were then washed again many times further with Milli Q water. 100 copepods were placed into a 30 ml glass vial containing 10 ml of 10% KOH and the samples were digested in a water bath at 60°C for 48 hours (Dehaut et al., 2016; Collicutt et al., 2019; Zheng et al., 2020). Following digestion, the samples were allowed to cool to ambient temperature before being vacuum-filtered using 0.22- μm Nitrocellulose Filter Paper (Millipore) and stored in covered Petri plates until detailed microscopic examination. The name of the station and the date of sample analysis was recorded on the Petri plate (Dehaut et al., 2016; Zheng et al., 2020). A powerful stereo zoom microscope was used to examine microplastic particles trapped on filter paper (OLYMPUS SZX16). The morphometrics (size, shape, and colour) of suspected particles of MPs were recorded and analysed using the image analysis software (Cell Sens software). For each station, MPs ingestion by copepods was determined by dividing the number of particles detected by the number of digested individuals and was expressed as no./individual copepod (Rashid et al., 2021). The chemical composition of MPs was determined using a confocal Raman spectrometer with AFM (WITec Alpha 300R, Germany) with the following setting: 532 nm excitation laser, 600 grooves/mm grating, and a 20 X objective with a 1s integration time. Wiley's Know It All Raman spectrum database for polymers and earlier papers were used to validate the resulting spectra. To avoid contamination from air-borne plastics and cross-contamination, all glassware and forceps were washed with distilled water before use, the workstation was sanitized before use, and each set of samples got a procedural control (10 ml of 10% KOH in an empty vial) (Zheng et al., 2020; Rashid et al., 2021).

2.3 Statistical analysis

To understand the influence of Thanneermukkom barrage (TB) on environmental parameters the nMDS analysis (euclidian distance matrix) was carried out to segregate the locations based on their resemblance in environmental parameters. This analysis was carried out for all the seasons sampled during this study. The environmental parameters were normalised before the analysis. The locations within the cluster have more similar characteristics, whereas the locations in different clusters represent dissimilar characteristics among them. To test the significance of forming the clusters, we used the SIMPROF permutation analysis (Leps and Smilauer, 2003). The statistical data analysis was performed using the XL-Stat Pro software package. One-way ANOVA was used to compare MPs ingested by copepods among the study locations. ANOVA test results were considered significant at p values < 0.05 . Correlation between the number of MPs and size was achieved with the Spearman correlation analysis.

3. Results

3.1. Physical variables

The seasonal opening and closing of TB shutters change the physicochemical properties of the KBW, and during the non-monsoon period (PSWM and NEM), the stagnant water upstream of the KBW becomes a region of various contaminants. The seasonal variation of temperature, salinity, and turbidity imposed by TB in the study region is presented in table 2. Temperatures in the study region varied seasonally and spatially, ranging from 27.3 °C to 32.8 °C (Figure 2; Table 2). The water column was warmer in PSWM (28.5 °C–32.8 °C) compared to the NEM (28.42 °C–30.3 °C) and the SWM (27.26 °C–30.92 °C). Spatially, the temperature increased from downstream to upstream stations (Table 2). A limnohaline to polyhaline level (from 0.1 to 21.01) of surface salinity was found and showing a significant temporal and spatial variation in the study region (Table 2). In all the locations, water column salinity was high during the PSWM, followed by the NEM and the SWM (Figure 3; Table 2). At the downstream locations, salinity was at polyhaline level at station 1 during the PSWM and mesohaline during the NEM. In station 2 salinity is at a mesohaline level during the PSWM and the NEM. In the KBW, mesohaline condition prevails in the midstream region during the PSWM and midstream to downstream regions during the NEM, whereas upstream locations exhibited oligohaline conditions during the PSWM. During SWM, the entire study region was in limnetic condition.

Overall, the salinity seasonally varied from limnetic to polyhaline (0.1 to 21.01) in the downstream, from limnetic to mesohaline levels (0.25 to 12.15) in the midstream, and from limnetic to oligohaline (from 0.08 to 4.9) in the upstream (Figure 3; Table 2).

3.2. Calanoid abundance and species distribution

TB plays a key role in the seasonal distribution and abundance of copepods in the KBW. Based on the distribution of the environmental parameters, the study locations were seasonally classified as different clusters. During the PSWM (closed condition of TB) the study locations were grouped into three clusters; locations 1 and 2 in cluster I (C I), locations 3 and 4 in cluster II (C II), and locations 5 and 6 in cluster III (C III). C I was in the downstream locations and characterised as polyhaline salinity (av. 23 ± 3), C II formed in the midstream locations and mesohaline salinity (av. 14 ± 2). C III was the upstream with oligohaline salinity (av. 2 ± 1.9) in KBW. During the SWM (opened condition of TB), the entire KBW was dominated with the freshwater (oligohaline), and the salinity was least, so that, there were no separate clusters of locations. During the NEM (partially open condition of TB), the entire locations were demarcated into three clusters same as PSWM. C I was mesohaline to polyhaline (av. 14 ± 2 salinity). C II was oligohaline to mesohaline (2-10 salinity). C III in the upstream was limnohaline (av. 0.3 ± 0.2 salinity). However, the abundance of copepods varied by the seasonal fluctuation of salinity distribution due to TB in the KBW. The environmental parameters NMDS plots overlapped with copepods abundance were shown in Figure 4.

In the present study, the total calanoid copepod abundance ranged from 107 to 1406 No. m^{-3} in the study region (Figure 4; Table 2). Spatially, in the downstream and upstream regions, copepod abundance was high during the NEM, followed by the PSWM and the SWM. In midstream, abundance was high during the PSWM and low during the SWM. During PSWM, abundance varied from 163 to 685 No. m^{-3} , with a maximum in the midstream. Copepod abundances were higher downstream during the SWM (107 - 443 No. m^{-3}) and the NEM (148 - 1406 No. m^{-3}). During all the seasons, a lower abundance of copepods was observed upstream oligohaline to limnetic condition (Figure 4; Table 2). Overall, the copepod abundance was found to be high in the mesohaline condition regardless of sampling regions and seasons. The seasonal distribution of calanoid copepods with their size range in the study region is presented in Table 3. A total of 26 calanoids were found and among them, *Acartia plumosa* and *Pseudodiaptomus*

annandalei were found throughout the KBW, regardless of the season. During the PSWM, *Acrocalanus gracilis*, *Acartia danae*, *Acartia plumosa*, *Paracalanus parvus*, *Bestulina similis*, and *Pseudodiaptomus annandalei* were dominant in the downstream, while *A. plumosa*, *B. similis*, *P. annandalei*, and *P. serricaudatus* were dominated in the midstream. In the upstream zone, *Acartiella gravelyi*, *Allodiaptomus mirabilipes*, *Heliodiaptomus cinctus*, and *Limnocalanus macrurus* were dominated. During the SWM, the most common copepods were *Acartiella gravelyi*, *Acartiella keralensis*, *Acartia plumosa*, *Allodiaptomus mirabilipes*, *Heliodiaptomus cinctus*, and *Limnocalanus macrurus*. During the NEM, *A. plumosa*, *P. annandalei*, and *A. gracilis* dominated in the downstream, *Acartia plumosa*, *Pseudodiaptomus annandalei*, *Allodiaptomus mirabilipes*, and *Acartiella keralensis* in the midstream and *Acartiella gravelyi*, *Allodiaptomus mirabilipes*, *Heliodiaptomus cinctus*, and *Limnocalanus macrurus* in the upstream location (Table 3).

3.3. Characteristics of MPs ingestion

MPs in copepods were acquired from all stations and seasons, except for stations 1 and 5 during the SWM. During this study, 1800 calanoid copepods were examined, revealing a total of 73 MPs. The PSWM gained the most MPs, followed by the NEM and SWM (Table 4). In the study area, the MPs in copepods varied from 0 to 0.13 no./ind. (Table 4). The MPs consumption by copepods in the three KBW zones ranged from av. 0.01 ± 0.01 to av. 0.11 ± 0.03 no./ind. (Figure 5). The MPs in copepods during the PSWM was found to be greater in the upstream (av. 0.11 ± 0.03 no./ind.) than in the downstream (av. 0.015 ± 0.007 no./ind.). During the SWM, the midstream had greater MPs (av. 0.035 ± 0.007 no./ind.) than the downstream (av. 0.015 ± 0.021 no./ind.) and upstream (av. 0.01 ± 0.014 no./ind.). During the NEM, the upstream had more MPs in copepods (0.075 ± 0.021 no./ind.) than the downstream (0.015 ± 0.007 no./ind.), similar to the PSWM. Copepods in the upstream showed higher MPs during the PSWM and NEM and lower MPs during the SWM (Figure 5). The differentiation showed that the numbers of MPs in copepods were significantly higher when TB was closed PSWM and NEM than when it was fully open during the SWM ($p < 0.05$).

The average ingestion of MPs among the copepods in the study area was av. 0.041 ± 0.035 no./ind. The MPs ingested by copepods belong to three morphotypes, such as fibres, fragments, and microbeads (Figure 6). The seasonal distribution of different types of MPs in copepods is

presented in Figures 7 and Table 4. During PSWM, copepods in downstream station 1 ingested only MP fibres, whereas, in station 2, both MP fibres and fragments were ingested. In the rest of the station along midstream and upstream, copepods were found to have ingested all three types of MPs. During the SWM, all three types of MPs were found only in station 3 in the midstream, fibre and microbeads were found in station 2 in the downstream and station 4 in the midstream, and also fibre and fragment types were found in station 6 in the upstream region. During the NEM, only microbeads were found at station 2, whereas fibre and fragments were found in stations 1 and 4. The remaining stations provided all three types of MPs (Table 4, Figure 7). In short, among the MPs, copepods ingested more fibres (54%), followed by microbeads (26%), and fragments (20%). The morphotypes of MPs in copepods showed that there was no significant seasonal difference in the KBW during this study period ($p>0.05$). The MPs ingested by the copepods, the colour blue (70%) was the dominant one, followed by black (14%), red (11%), and green (5%). MPs fibres were present in all four colours: fragments in blue, black, and red, while microbeads only appeared in blue. The comparison of the colour of MPs and copepods ingestion was significant and the blue coloured MPs proportion was found higher in copepods ($P<0.05$).

The average length of MPs found in the copepods ranged from $12 \pm 3.3 \mu\text{m}$ (microbead) to $71.6 \pm 29.8 \mu\text{m}$ (fibre) and their average length in three seasons is presented in Figure 8. During the PSWM, the length of the fibre, fragment, and microbead was $\text{av.}53.21 \pm 19.04 \mu\text{m}$, $\text{av.}47.98 \pm 18.82 \mu\text{m}$, and $\text{av.}13.46 \pm 3.61 \mu\text{m}$, respectively. During the SWM, it varied from $\text{av.}71.65 \pm 29.79 \mu\text{m}$ for fibre, $\text{av.}59.78 \pm 25.8 \mu\text{m}$ for the fragment, and $\text{av.}20.72 \pm 5.1 \mu\text{m}$ for microbead. During the NEM, the length was $\text{av.}59.67 \pm 19.56 \mu\text{m}$ for fibre, $\text{av.}40.53 \pm 10.08 \mu\text{m}$ fragment, and $\text{av.}11.99 \pm 3.36 \mu\text{m}$ for the microbead (Figure 8). However, the overall length of MPs in the study ranged from $8.09 \mu\text{m}$ to $108.17 \mu\text{m}$ (Table 4) with an average length of the fibre $\text{av.}58.67 \pm 21.88 \mu\text{m}$, the fragment $\text{av.}47.07 \pm 17.1 \mu\text{m}$ and the microbead $\text{av.}14.06 \pm 4.65 \mu\text{m}$. ANOVA results showed that there was no significant seasonal difference ($p>0.05$) was observed in a comparison of the length of MPs and the ingestion by copepods.

The spectra of selected MPs were retrieved using Micro-Raman spectroscopy and confirmed by comparing them with the spectral library. Four types of polymers were present in this study (Figure 9), Polypropylene (PP), Low-density polyethylene (LDPE), Nylon (PA) and Polystyrene

(PS) in this PP and LDPE were predominant. The spectral range for PP (810 – 2955 cm^{-1}), LDPE (1061 – 2881 cm^{-1}), Nylon (1131 – 2898 cm^{-1}) and PS (619 – 3052 cm^{-1}) are understood (Figure 9).

4. Discussion

This baseline study in the KBW showed that the copepods ingest MPs, which corroborates many studies from other parts of the world (Desforges et al., 2015; Sun et al., 2017; Sun et al., 2018a, 2018b; Kosore et al., 2018; Zheng et al., 2020; Amin et al., 2020; Taha et al., 2021; Zheng et al., 2021; Aytan et al., 2021). Studies have shown that MPs in the aquatic environment may either be primary (e.g., microbeads in toothpaste, exfoliates in personal care products, beads used for drug delivery) or secondary (fragmentation of larger plastic particles) and they attain a wide variety of shapes (pellets, fibres, irregular fragments, films, granules, and foam), sizes and colours (Hidalgo-Ruz et al., 2012; Wright et al., 2013). It is quite logical that due to their smaller size, lightweight and persistent nature, MPs are bioavailable to a wide range of organisms inhabiting both the pelagic and benthic realms (Moore, 2008; Wright et al., 2013; Nikki et al., 2021).

During the PSWM, a significant salinity gradient was seen in the KBW from downstream to upstream stations (Figure 3, Table 2); the downstream was euhaline, the midstream was mesohaline, and the upstream was limnetic (Figure 3, Table 2) (Jyothibabu et al., 2006; Arunpandi et al., 2020). On the other hand, due to heavy rainfall and river influx during the SWM, limnetic conditions persisted throughout the KBW. During the NEM, the salinity regained its dominance with a euhaline/mesohaline salinity in the downstream and the limnetic situation in the upstream, a spatial trend very similar to the PSWM. This seasonal change in the salinity distribution in the KBW is due to the low river influx during the PSWM and NEM and significantly higher river influx during the SWM (Madhupratap, 1987; Jyothibabu et al., 2006). As mentioned before, the Thanneermukkom barrage (TB) played a remarkable role in the salinity distribution in the KBW during the PSWM, also the present study during PSWM, when the shutters of the TB were fully closed, it maintained a limnetic condition upstream, which otherwise would have been mesohaline in nature (Haridevan et al., 2015; Anjusha et al., 2018; Arunpandi et al., 2020). This study showed that the artificial flow restriction and the resultant

stagnancy caused by the TB upstream have a noticeable impact on the composition of copepods as well as the MPs they ingested.

4.1. Calanoid abundance and composition

The abundance of calanoid copepods in the study region ranged from 107 (upstream) to 1406 No. m⁻³ (downstream). During PSWM, abundance was high in the midstream region (av. 639 ± 47 No. m⁻³) compared to downstream (av. 418 ± 36 No. m⁻³) and upstream (av. 172 ± 13 No. m⁻³). Compared to PSWM and NEM, low abundance was observed in all three zones during SWM. These results corroborate previous studies on the spatial distribution of zooplankton in the KBW (Madhupratap, 1987; Wellershaus, 1974; Arunpandi et al., 2020, 2021a). During SWM, abundance was higher in the downstream location than in the other two. This may be due to the high flushing rate in the KBW, which brings upstream copepods towards the downstream (Madhupratap, 1987; Wellershaus, 1974). Overall, high copepod abundance was present in stations having mesohaline levels (5–18) of salinity (Arunpandi et al., 2020, 2021 a). A total of 26 calanoid copepods were identified in this study and the majority of them were distributed downstream and upstream. *Acartia plumosa* and *Pseudodiaptomus annandalei* were found throughout the KBW, regardless of the season. This may be due to the wide salinity tolerance (euryhaline nature) of these copepods, even though they show higher abundance in the mesohaline zone (Madhupratap, 1979; Arunpandi et al., 2020). The construction of TB has changed the hydrography of KBW, which resulted in significant changes in the distribution and abundance of copepods in KBW, especially in non-monsoon seasons. Several authors have reported the salinity tolerance of copepods in KBW (Madhupratap and Haridas, 1975; Madhupratap, 1979; Martin Thompson, 1991; Tranter and Abraham, 1971; Arunpandi et al., 2021a). During SWM, mesohaline to polyhaline salinity favoured the dominance of *Acartia plumosa*, *A. centura*, *A. danae*, *A. erythraea*, *A. southwelli*, *Acrocalanus gracilis*, *Paracalanus parvus*, and *Labidocera acuta* in the downstream region. Copepods, *A. plumosa*, *B. similis*, *P. annandalei*, and *P. serricaudatus* dominated midstream due to the mesohaline salinity. On the other hand, oligohaline salinity in the upstream caused the dominance of *Acartiella gravelyi*, *Allodiaptomus mirabilipes*, *Heliodiaptomus cinctus*, and *Limnocalanus macrurus*. During the SWM, the entire study area was limnetic, which resulted in the dominance of low saline species *A. gravelyi*, *A. keralensis*, *A. plumosa*, *A. mirabilipes*, *H. cinctus*, and *L. macrurus*. During the NEM, mesohaline salinity in the downstream favoured the dominance of *A. plumosa*, *P.*

annandalei and *A. gracilis*, while oligohaline to mesohaline salinity in the midstream favoured *Acartiella keralensis*, *Acartiella gravelyi*, *Acartia plumosa*, *Allodiaptomus mirabilipes*, and *Psuedodiaptomus annandalei*. During the NEM, limnohaline to oligohaline salinity was present upstream which favoured the dominance of *A. gravelyi*, *A. mirabilipes*, *H.cinctus* and *L. marcrurus*.

4.2. Characteristics of MPs in copepods

High MPs concentrations in the surface waters of the KBW at various times of the year have been documented (Suresh et al., 2020; Nikki et al., 2021). These data strongly suggest that MPs are always bioavailable to all species living in the KBW. It has also been observed that MPs are present in sediment as well as higher trophic level creatures in the KBW (Sruthy and Ramasamy, 2017; Daniel et al., 2020; Devi et al., 2020; Suresh et al., 2020; Nikki et al., 2021). This study found that calanoids in KBW consume MPs and there have been reports of comparable incidents from throughout the world, a summary of which is shown in Table 5. We recently showed MPs in copepods from the coastal waters of India's west coast in another research (Rashid et al., 2021). It is crucial to note that only calanoid copepods ($> 500 \mu\text{m}$) were chosen for this study, and copepods from the orders Cyclopoids and Harpacticoid were excluded for the following reasons. We looked for MPs in all three copepod orders (calanoida, cyclopoida, and harpacticoida) in trial research, but we only found them in calanoids. Their smaller body size (av. $0.72 \pm 0.12 \text{ mm}$) most likely inhibit MPs intake as compared to calanoids (Buathong et al., 2020; Taha et al., 2021). Except for station 1 and station 5 during SWM, MPs were recovered from all the stations, regardless of seasons, and the MPs in copepod varied from 0 to 0.13 no./ind. with average ingestion of $0.041 \pm 0.035 \text{ no./ind.}$ This ingestion value is higher than that reported from calanoids in the Terrenganu estuary ($0.02 \pm 0.02 \text{ no./ind.}$) (Taha et al., 2020) and the Black Sea ($0.024 \pm 0.020 \text{ no./ind.}$ for *Calanus euxinus*; $0.008 \pm 0.006 \text{ no./ind.}$ for *Acartia clausi*) (Aytan et al., 2021), but significantly lower than that reported from upper Thailand Gulf (av. $0.45 \pm 0.04 \text{ no./ind.}$) (Buathong et al., 2020). Ingestion of MPs in KBW copepods is comparable to those observed in Kochi coastal waters [$(0.03 \pm 0.01 \text{ no./ind.})$; Rashid et al., 2021] (Table 5). Variations in MPs ingestion are expected in estuaries due to varying hydrographic environments, which are frequently affected by hydrodynamics, sample site location, and degrees of human disturbance (Nazarnia et al., 2020; Peters and Bratton, 2016).

The TB remains fully open during the SWM, partially open during NEM and fully closed during the PSWM, which influence the number of MPs in copepods. During the PSWM, the average number of ingested MPs in copepods was significantly greater in the upstream (av. 0.11 ± 0.028 no./ind.) than in the downstream (av. 0.015 ± 0.007 no./ind.). The prolonged closure of TB during PSWM increases the residence time of the lake. This causes an accumulation of a large number of MPs in the upstream region, which comes from nearby houses, factories, wastewater treatment plants, municipal sewage, agricultural discharge, tourism activities, fishing and this may be the reason for the higher ingestion of MPs in copepods. Many copepods have mechano and chemosensory receptors (Kiorboe, 2011) and have the ability to distinguish the quality of food, choosing natural foods rather than artificial ones (Cole et al., 2016; Harrison et al., 2011). The long residence of water during PSWM causes biofilm formation on MPs, which generates chemosensory responses more similar to food items (Lobelle and Cunliffe, 2011), making them bioavailable to organisms. Some previous studies have reported that copepods ingest more aged MPs than virgin ones (Vroom et al., 2017; Procter et al., 2019). This also may be a reason for higher MPs accumulation in copepods during PSWM. Among the three seasons, lower ingestion was observed during the SWM, when TB was in fully open condition. Because of high runoff during this season, washes all debris into the nearby Arabian Sea, MPs was found less available in surface water, which could be the reason behind the lower ingestion of MPs observed during this period (av. 0.01 ± 0.014 no./ind.). During the NEM period, the TB was only partially open, which caused a certain level of stagnancy in the upstream, complemented with river discharge caused a relatively high concentration of MPs in the upstream (av. 0.075 ± 0.021 no./ind.) compared to the downstream (av. 0.015 ± 0.007 no./ind.).

The present study showed that the overall size of ingested MPs ranged from $8.09 \mu\text{m}$ to $108.17 \mu\text{m}$ (Table 4). This range was smaller than the reported size ranges in some earlier studies (Table 5). The larger size was for fibre and the smaller size was for microbeads. Also, the length of different types of MPs varied in the three zones of KBW during different seasons (Figure 8). Compared to PSWM and NEM, SWM had a greater length for all types of MPs. This could be due to the presence of large-sized MPs in surface water carried by the enhanced influx of the rivers and canals, similar to the observations from the Bohai Sea, in which zooplankton ingested large-sized MPs during the rainy season (Zheng et al., 2020). In the study region, the average size of ingested MPs by calanoid copepods was av. $58.67 \pm 21.88 \mu\text{m}$ (Fibre), av. 47.07 ± 17.1

µm (Fragment) and av. 14.06 ± 4.65 µm (microbead). The observed values in this study were lower than those reported in earlier studies (Table 5). The average fibre length obtained in this study (av. 58.67 ± 21.88 µm) was shorter than that obtained from calanoid copepods in the upper Gulf of Thailand Sea [(av. 458.6 ± 311.3 µm) Buathong et al., 2020] and Terengannu coastal waters [(313 µm); Amin et al., 2020]. Similarly, the length of fragment obtained in this study (av. 47.07 ± 17.1 µm) was shorter than that reported in two black sea species [(av. 121 ± 128 µm, *Acartia cluasi*), and (av. 66 ± 43 µm, *Calanus euxinus*) Aytan et al., 2020]. It is, however, comparable to Terengannu coastal waters [(56.3µm)] (Amin et al., 2020). The sizes of MPs ingested by calanoid copepods correspond to the size range of their natural prey (phytoplankton, microzooplankton, and marine snow/aggregation). When considering the shape of MPs, fibres (54%) were the dominant type, followed by microbeads (26%) and fragments (20%). Fibres are reported to be the most prevalent type of MPs debris in the natural environment (Browne et al., 2011; Cesa et al., 2017).

Fibres were found to be the most common MPs ingested by copepods in the East China Sea (Sun et al., 2018a), Yellow Sea (Sun et al., 2018b), Bohai Sea (Zheng et al., 2020), Jiaozhou bay (Zheng et al., 2021) and Terengganu estuary and offshore (Taha et al., 2021). However, fragments were the most common MPs ingested by the species *A. clausi* and *C. euxinus* of the Southeastern Black Sea (Aytan et al., 2020), and by calanoid and cyclopoid copepods in the South China Sea (Amin et al., 2020). All types of MPs in copepods observed in the present study were also reported recently from the coastal waters of Kochi (Rashid et al., 2021). Previous studies in the KBW showed that fibres were the major type of MPs in water and within biota (Table 1), which mainly originate from synthetic textiles, discharge from washing machines, household sewage, industrial effluents, fishing gear such as rope and net, tourism activities. Fragments of MPs mainly originate from the breakdown of single-use plastic covers, plastic bags and food containers, whereas, microbeads MPs originate from personal care products, toothpaste, and discharge from wastewater treatment plants (Fendall and Sewell, 2009; Browne et al., 2011; Cole et al., 2013). Botterell et al. (2020), showed that copepods (*Acartia tonsa* and *Calanus helgolandicus*) ingest all three types (fibre, fragment, and microbead) of MPs and suggested that, future impact studies should also consider the environmentally relevant MPs (fibre, fragment) as well. Four coloured MPs were obtained in this study; blue (70%) was the dominant one, followed by black (14%), red (11%) and green (5%) and similar results are

recorded in many earlier studies as well (Table 5). Blue has been reported as the dominant colour of MPs in copepods from the Bohai Sea (Zheng et al., 2020), Thailand's upper gulf (Buathong et al., 2020), and Kochi's coastal waters (Rashid et al., 2021). Blue coloured MPs has also been reported from many other biotas in the KBW in earlier studies (Table 1). Coloured microplastics are considered to be more harmful to marine life than clear microplastics, as the chance of being swallowed by aquatic animals, followed by a trophic transfer, is high in the case of coloured microplastics (Qu et al., 2018; Au et al., 2017).

Raman spectra of the analyzed MPs samples revealed that four types of polymers (PP, LDPE, PA and PS) are being known to be ingested by the copepods in the KBW (Figure 9). These results are quite comparable to those reported from the water and biota of the KBW (Table 1). Polypropylene and polyethylene-based plastics are dominant in the marine environment. PP is widely used to make plastic containers, food packaging, pipes, rope, drinking straws, bottle caps etc. (Andrady and Neal, 2009). Ziplock covers, toys, squeeze bottles, garbage and grocery bags are all made from LDPE (GESAMP, 2015). Nylon (Polyamide) is used in textiles, ropes, toothbrush bristles, fishing lines, films for food packaging etc. (Barboza et al., 2019). CD and DVD cases, single-use disposable cutlery, trays, disposable cups and plates are all made of PS (Andrady and Neal, 2009; GESAMP, 2015; Barboza et al., 2019).

4.3 Implication of MPs in copepods in the food web

The study demonstrated for the first time that the calanoid copepod community in KBW consumes a variety of MPs types and sizes regardless of season or location. Several experimental studies on copepods found that MPs ingestion harms feeding (Cole et al., 2013, 2015; Coppock et al., 2019; Yu et al., 2020), growth (Jeong et al., 2017; Zhang et al., 2019), development (Yu et al., 2020), reproduction (Lee et al., 2013; Cole et al., 2015; Yu et al., 2020), and survival (Lee et al., 2013; Kaposi et al., 2014; Cole et al., 2015; Yu et al., 2020; Shore et al., 2021). Setälä et al., (2014) suggest that MPs enter aquatic food webs primarily through zooplankton ingestion and could therefore transfer to higher trophic levels. Several authors found MPs trophic transfer; Setälä et al., (2014) demonstrated that a polystyrene sphere (10 µm) can be transported trophically from mesozooplankton (Copepod and Polychaete larva) to a mysid shrimp (*Mysis relicta*). Athey et al., (2020) observed the trophic transfer of 10-20 µm LDPE microbeads from unicellular tintinnids (*Favella* spp.) to larval inland silversides (*Menidia beryllina*). When

Aurelia coerulea ephyrae were exposed to aged microbeads and *Artemia nauplii*, the ephyrae consumed 35 times more microbeads via trophic transfer than by direct consumption (Sucharitakul et al., 2021). Costa et al. (2020) found a comparable trophic transfer of polyethylene microspheres (1–5 µm) between copepods (*Tigriopus fulvus* nauplii) and jelly fish (*Aurelia* spp). In addition, another example in lab conditions showed MPs transmission up the food chain; four species from the primary producer (algae) to the end-consumer (fish) were involved (Chae et al., 2018).

Wang et al., (2021) found an increased quantity of MPs in the upper trophic level (sandworm to fish) in field research in the Liaohe estuary, China . Similarly, MPs contamination in fin and shellfish in KBW was higher in earlier research than in copepods in this investigation. Because copepods are a key food source for them, trophic transfer from copepods to higher trophic levels in the KBW is extremely likely, and it may eventually reach humans. A schematic of the various sources and transfer pathways of MPs in the KBW has been presented in Figure 10. Earlier research found that KBW is contaminated with toxic chemicals, and the higher surface area to volume ratio of MPs allows them to absorb it and harm the health of consuming animals (Syberg et al., 2017; Bellas and Gil, 2020). Copepods also contribute to the nutrient cycle by ingesting and then repackaging particulate organic materials into dense faecal pellets with high sinking velocities (Turner, 2002). According to recent laboratory investigations, MPs in copepod faecal pellets have the potential to function as vectors for the vertical transmission of MPs to benthic species (Cole et al., 2016; Coppock et al., 2019; Shore et al., 2021). Because the typical depth of KBW is roughly 4 m, faecal pellet-laden MPs may reach the bottom quickly, allowing bioavailability to benthic inhabitants. All of these possibilities are alarming and may have an impact on ecosystem functioning, which must be thoroughly examined.

5. Conclusion

This study investigated MPs in calanoid copepods in the KBW and how a man-made saltwater barrage, TB, affected their intake on a seasonal basis. It showed that MPs exist in calanoids in the KBW regardless of season or location, with a clear seasonal and geographical difference. The average intake in the study locations (0.041 ± 0.035 no./ind.) was greater than any previously reported values for calanoid copepods. MPs ingested by copepods were mostly fibres (54%), followed by microbeads (26%), and fragments (20%). The size range of ingested MPs

(8.09 μm to 108.17 μm) is within the range of their feed size in the KBW. The closed shutters of the TB and the stagnancy induced in the upstream during the PSWM and the NEM revealed a substantial increase in MPs in calanoids during the PSWM (av. 0.11 ± 0.028 no./ind.) when TB shutters were entirely closed compared to the SWM (av. 0.01 ± 0.014 no./ind.) when TB shutters were fully opened. Because copepods are at the base of the food chain, ingested MPs with the ability to adsorb toxic chemicals can be transferred to higher trophic levels and finally reach humans. This study also calls for the scientific operation of the TB's shutters to avoid long-term stagnation of the KBW's upstream to decrease MPs accumulation via the food chain.

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Credit authorship contribution statement

C.P. Rashid: Conceptualization, Methodology, Data curation, Writing - original draft, Visualization, Funding acquisition. R. Jyothibabu: Conceptualization, Methodology, Data curation, Visualization, Writing - review & editing, Supervision, Funding acquisition. N. Arunpandi: Conceptualization, Methodology, Visualization, Formal analysis. S. Santhikrishnan: Methodology, Visualization. V. Vidhya: Methodology, Visualization. S. Sarath: Methodology, Visualization. M. Arundhathy: Data curation, Visualization. K. T. Alok: Data curation, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request through the Director, CSIR-National Institute of Oceanography, or the Scientist-in-Charge, Regional Centre Kochi.

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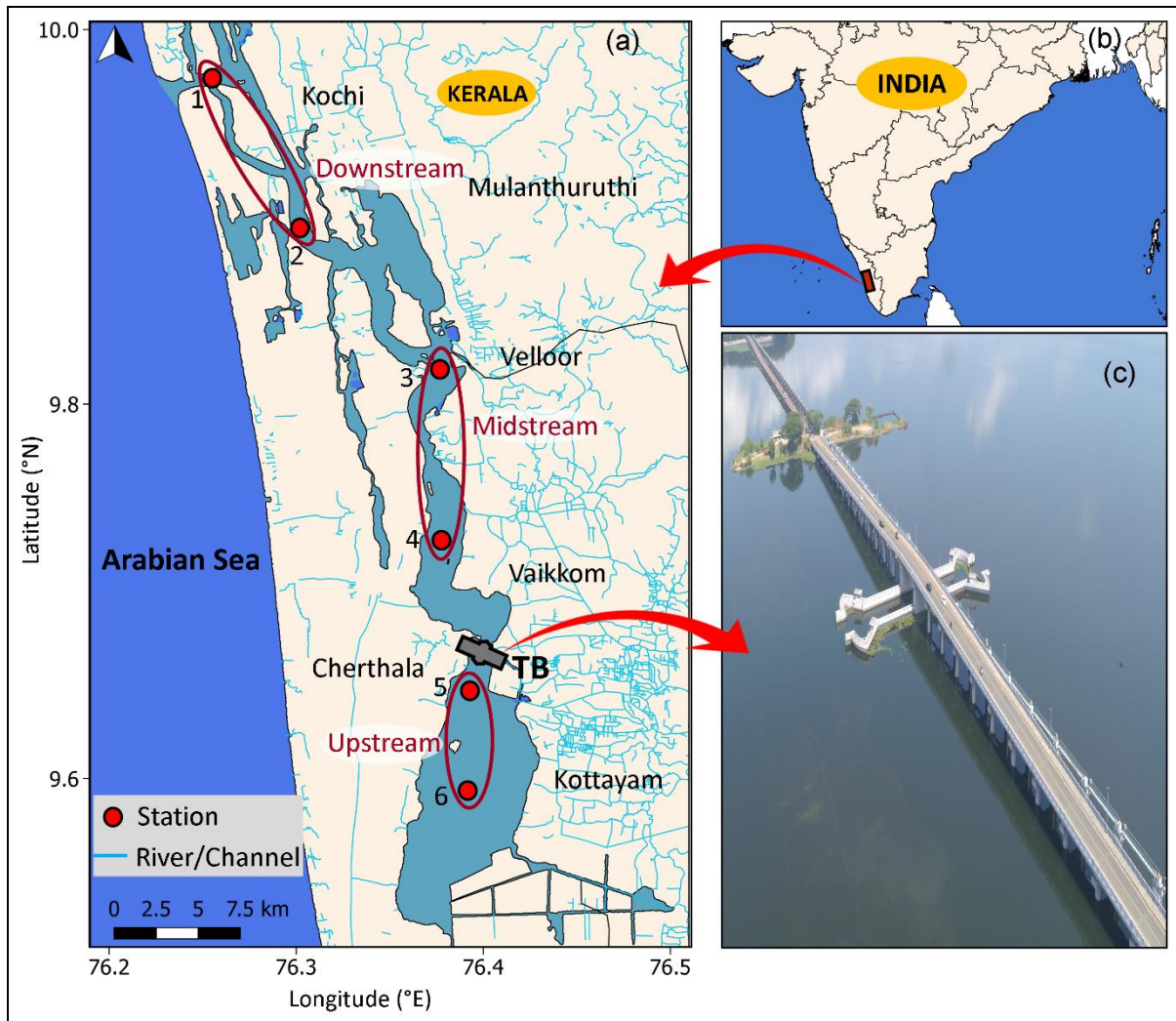


Figure 1- (a and b) Sampling region in the Kochi backwaters (KBW), red filled circles represent the sampling locations and (c) TB - Thanneermukkom saltwater barrage.

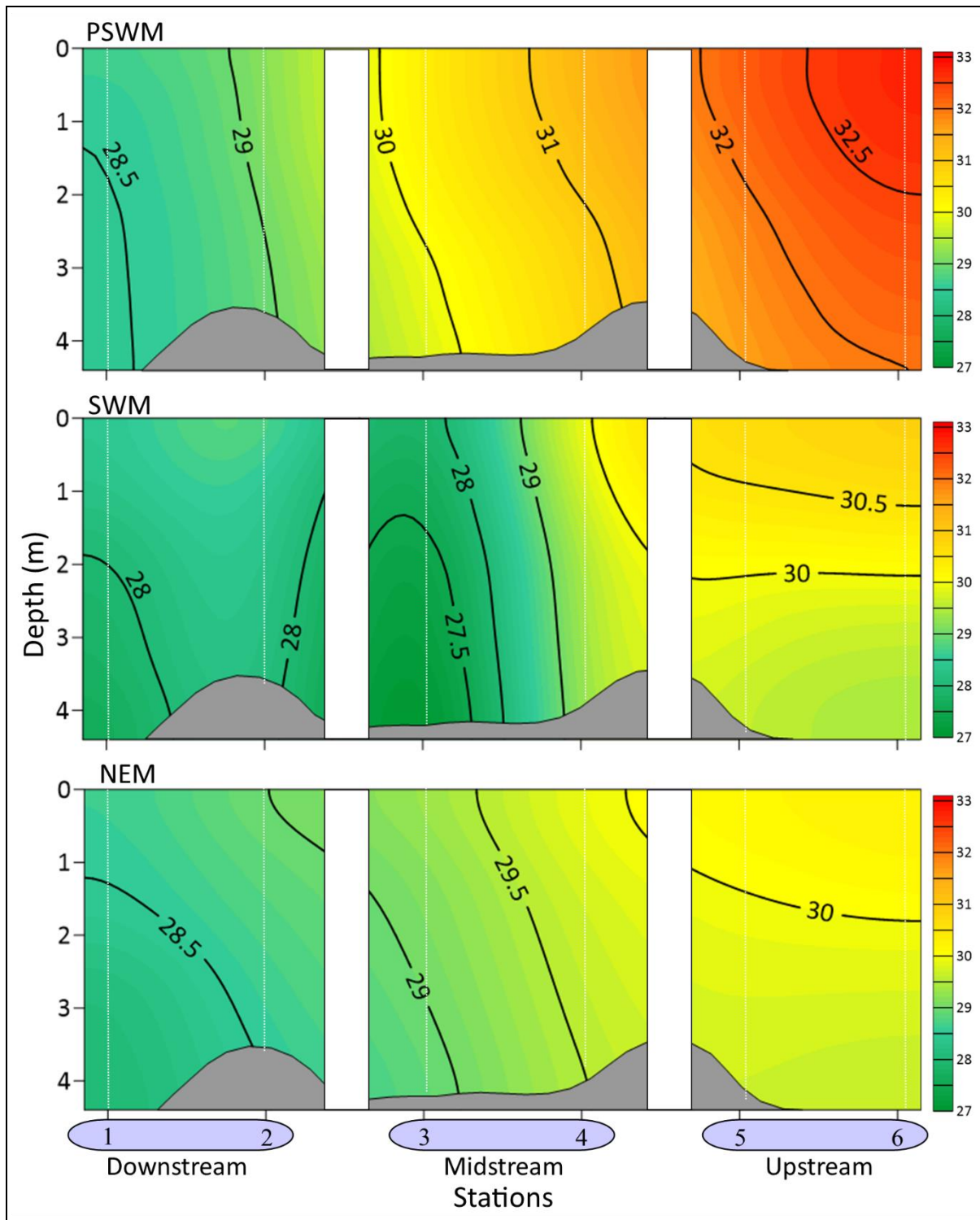


Figure 2- Vertical distribution of temperature in the sampling locations in the Kochi backwaters.

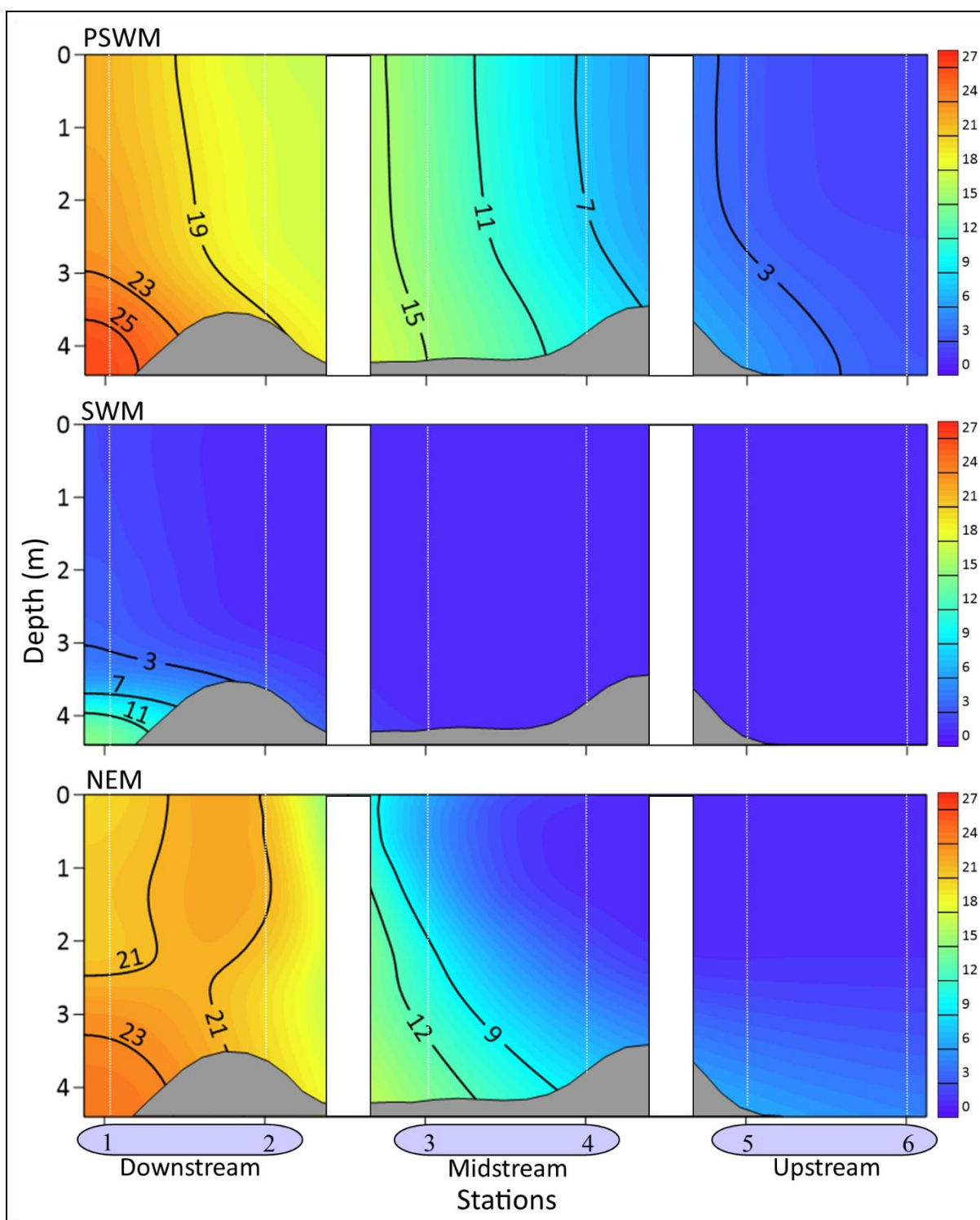


Figure 3- Vertical distribution of salinity in the sampling locations in the Kochi backwaters.

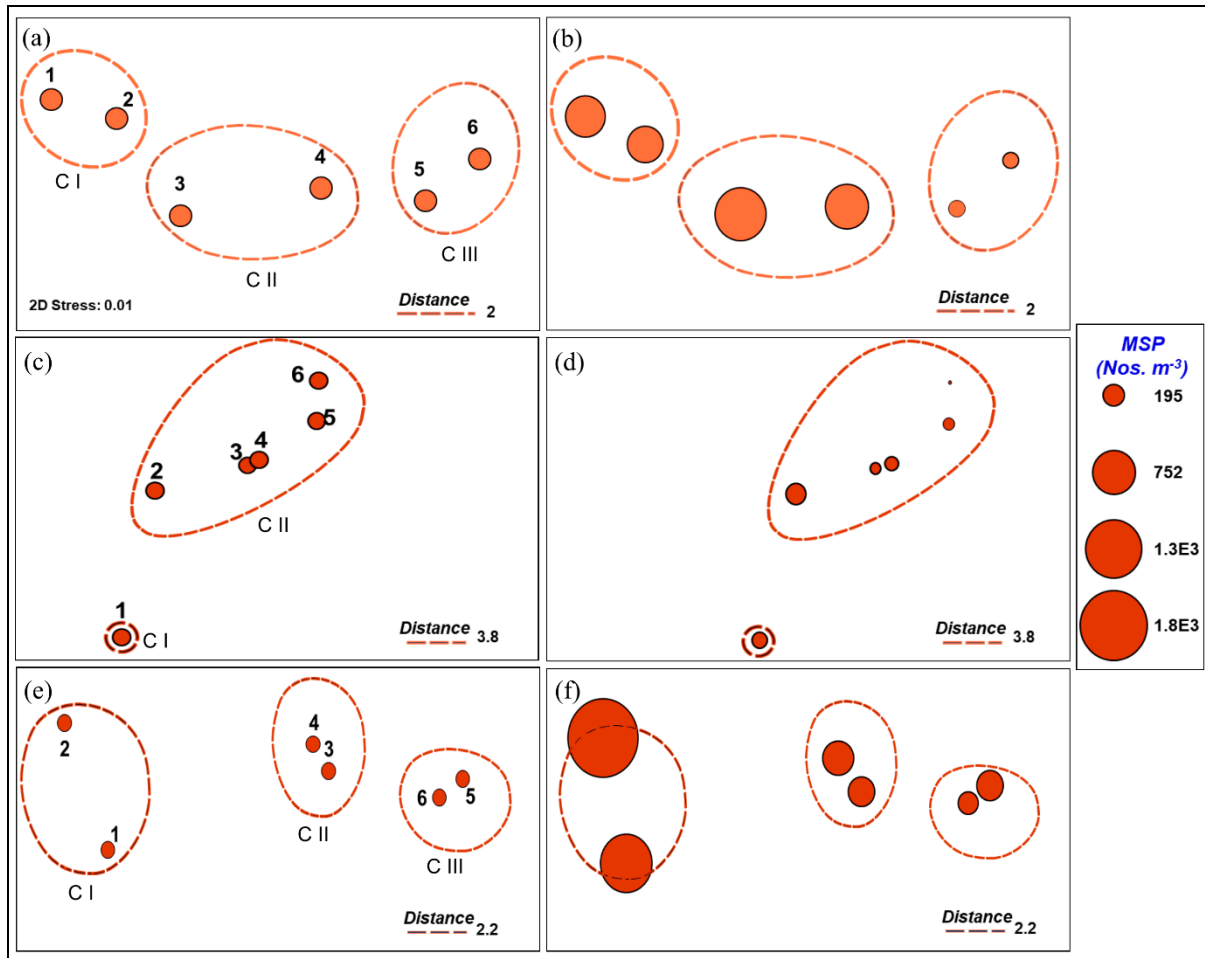


Figure 4- Cluster and NMDS plots presenting the separation of locations based on the distribution of environmental variables in the KBW during (a) Pre-Southwest Monsoon (PSWM), (c) Southwest Monsoon (SWM) and (e) Northeast Monsoon (NEM). Subsequent panels (b, d and f) show environmental variables NMDS plots overlaid with bubbles of mesozooplankton (MSP)/ copepods abundance.

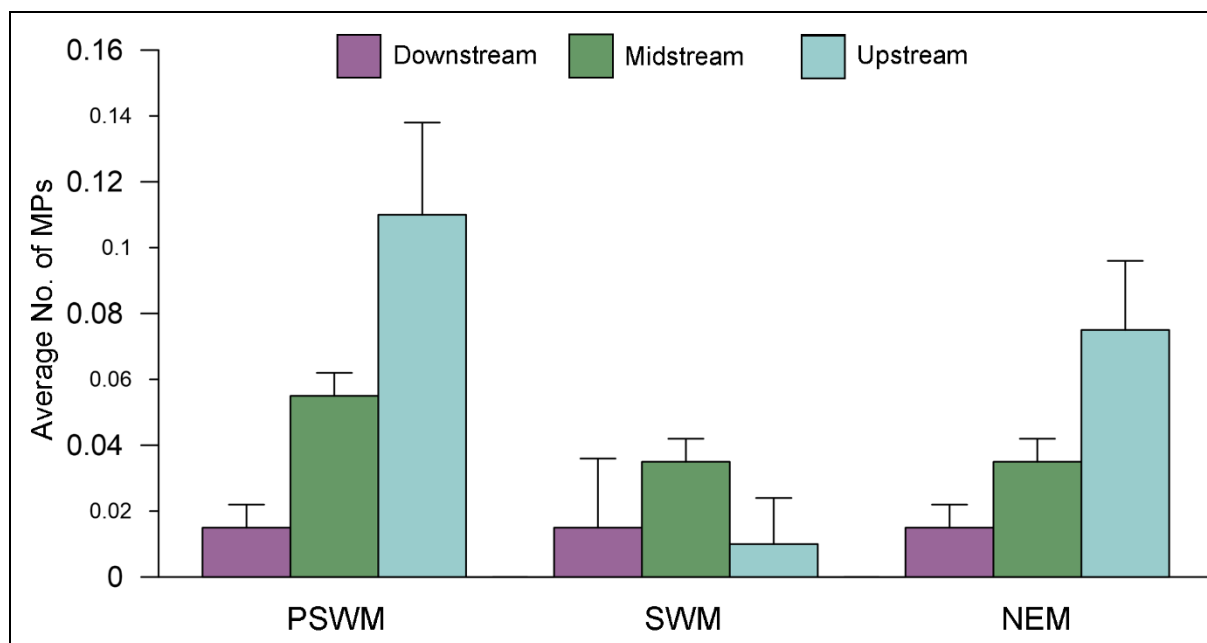


Figure 5- Average number of MPs in calanoid copepods in the three zones of KBW during different season. PSWM- Pre-Monsoon, SWM- Southwest Monsoon, NEM- Northeast Monsoon.

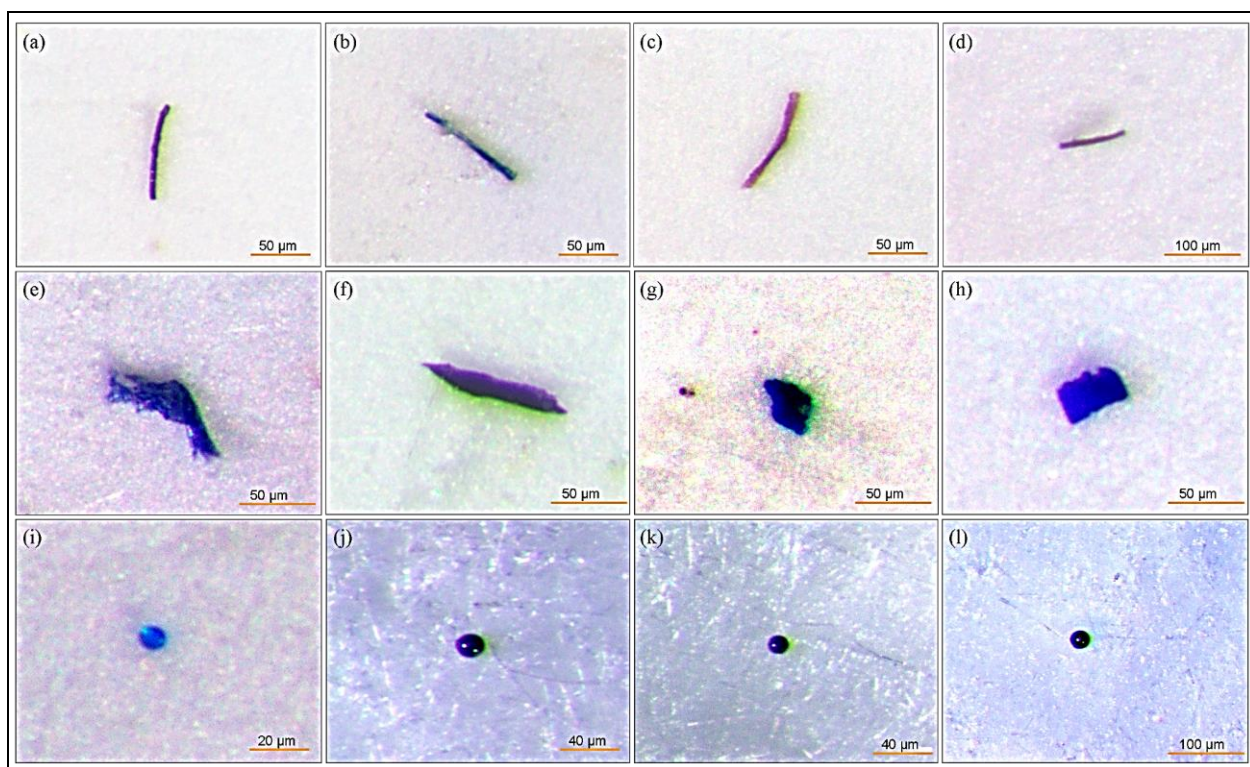


Figure 6- Microplastics ingested by copepods. (a-d) fiber, (e-h) fragment, (i-l) Microbeads

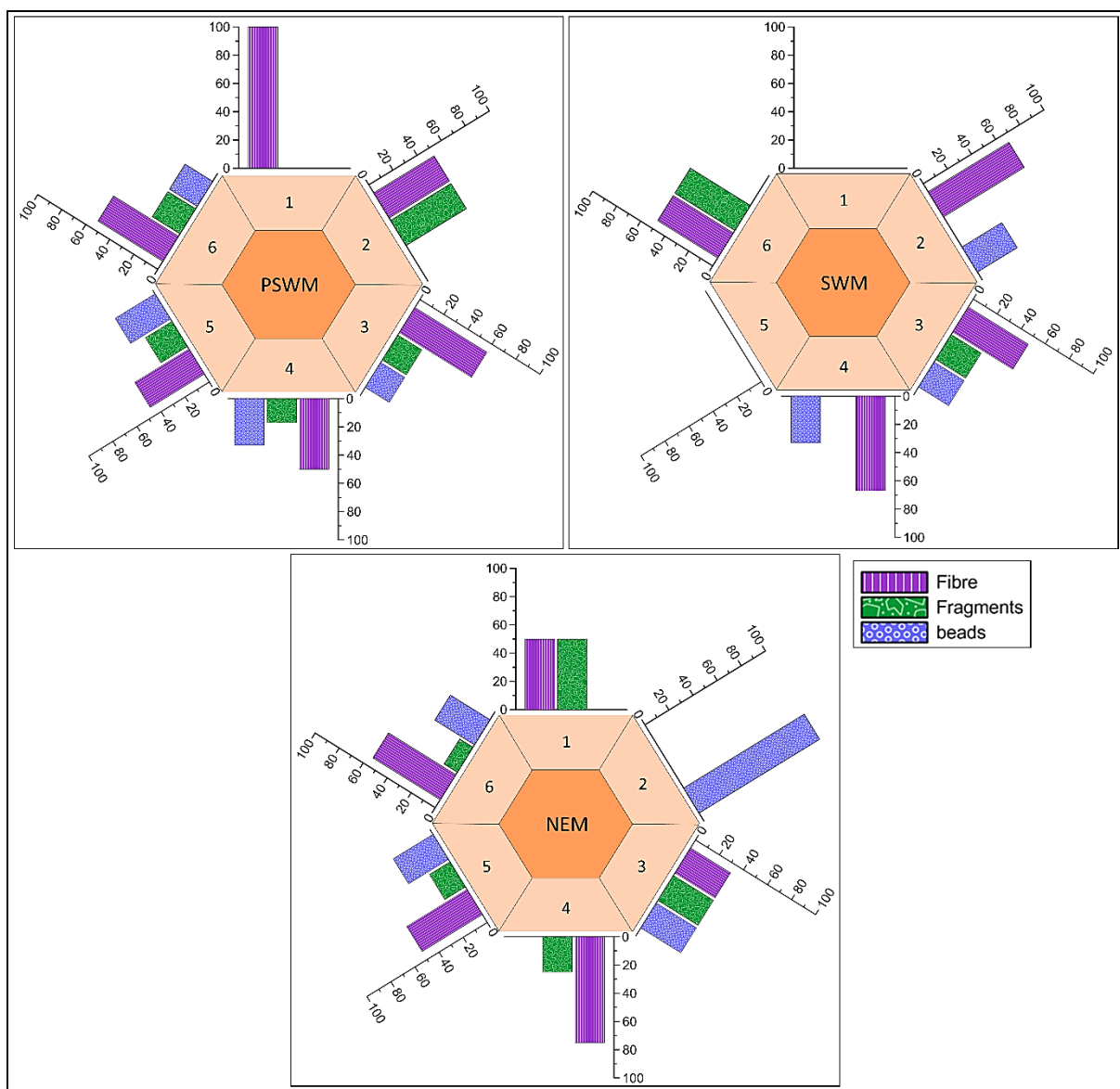


Figure 7- Percentage contribution of Microplastic (MPs) in the six locations in the Kochi backwaters. PSWM- Pre-Monsoon, SWM- Southwest Monsoon, NEM- Northeast Monsoon.

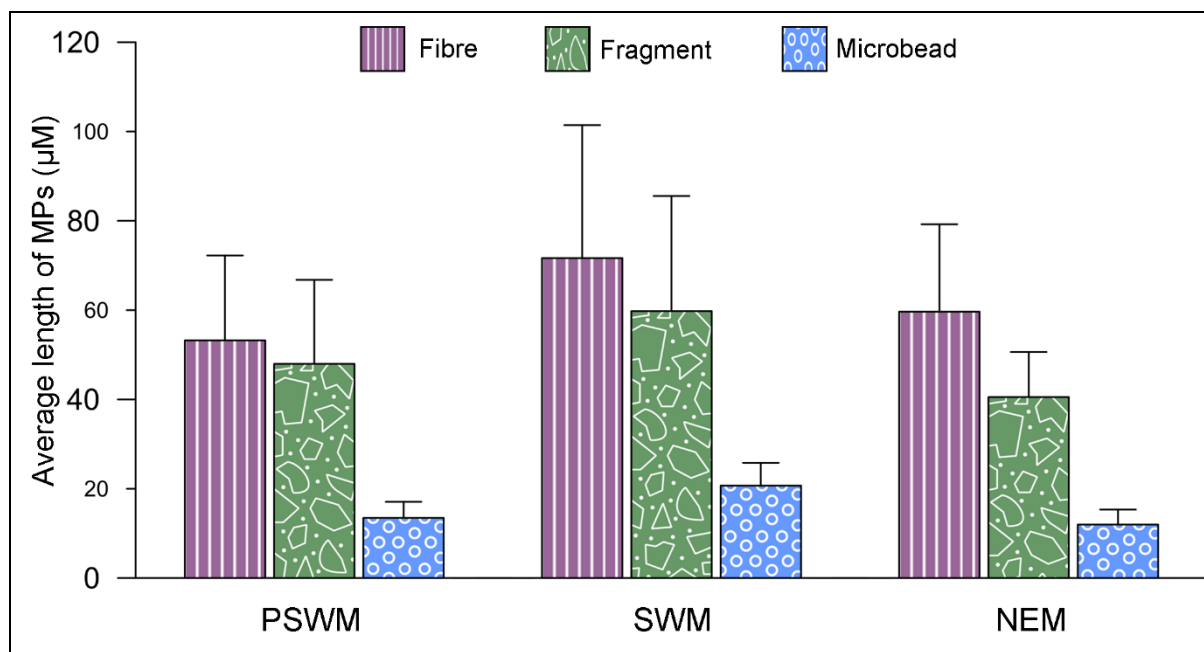


Figure 8- Seasonal average length of different types of MPs ingested by copepods during different seasons. PSWM- Pre-Monsoon, SWM- Southwest Monsoon, NEM- Northeast Monsoon.

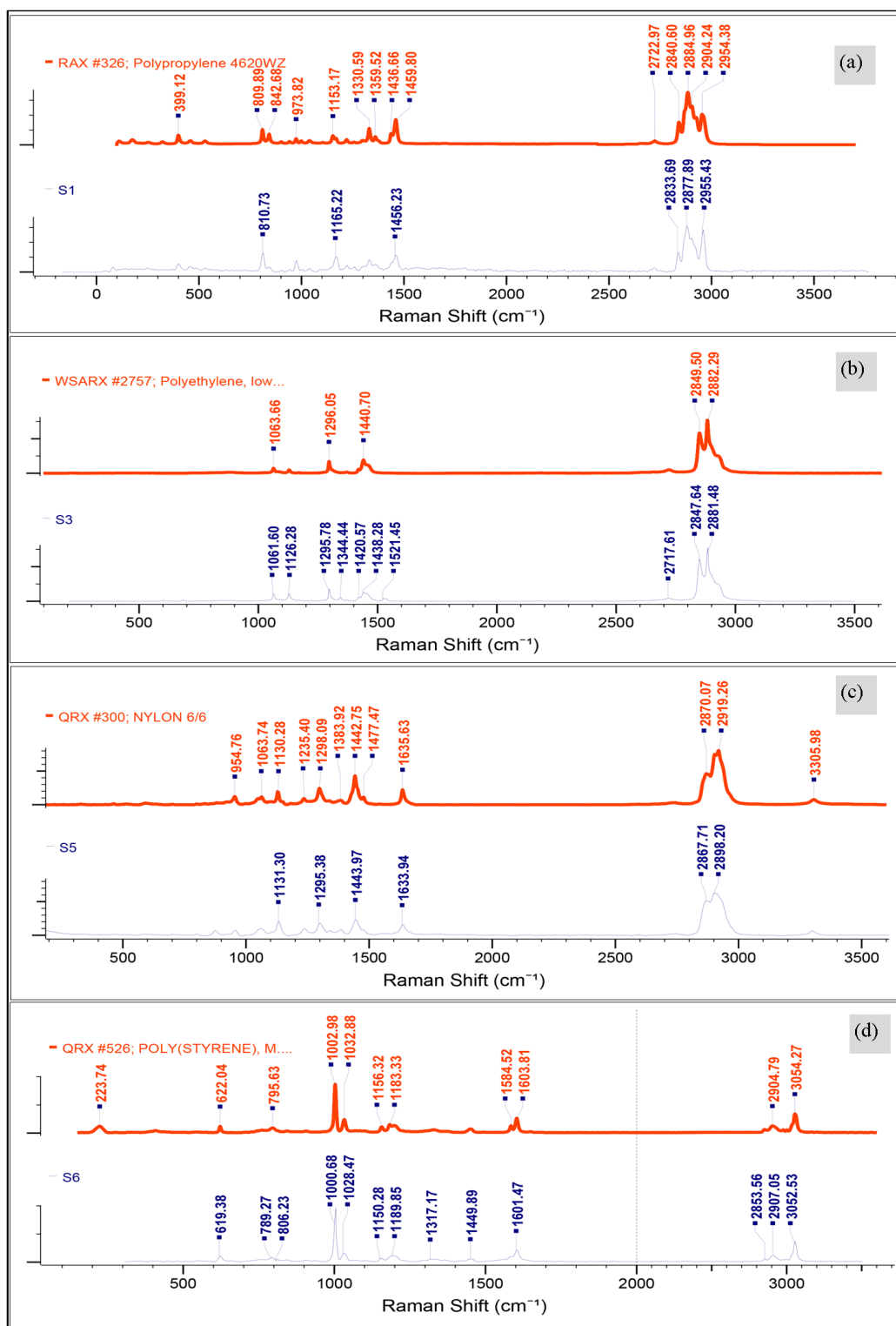


Figure 9- The spectra of four types of polymers [(a) Polypropylene, (b) Low density polyethylene, (c) Nylon and (d) Polystyrene] were retrieved using the micro-Raman spectroscopy and confirmed by comparing with reference spectrum (red colour line).

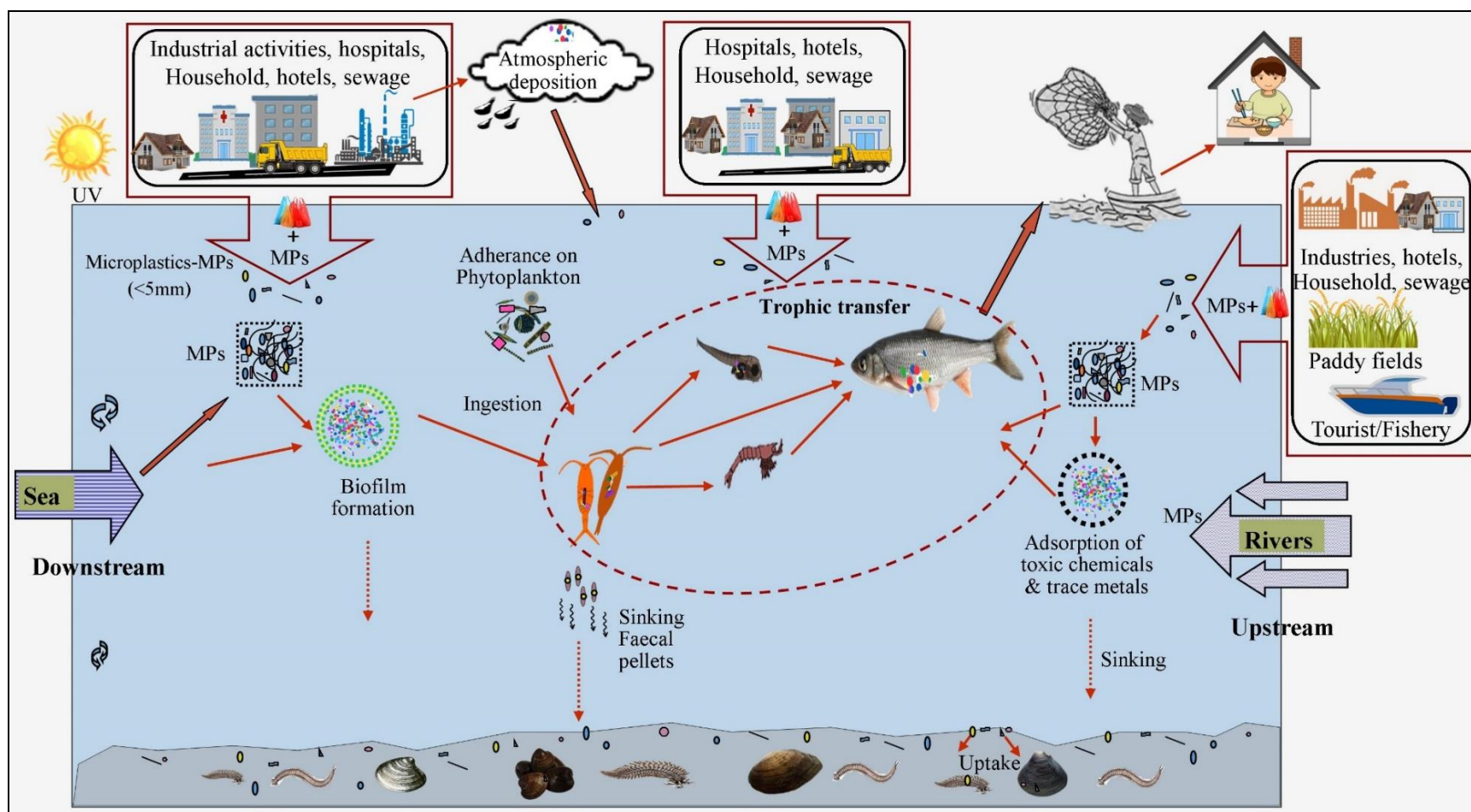


Figure 10- showed the schematical representation of transfer pathway of MPs in the system and trophic transfer of MPs from plankton food chain to top consumer humans.

Sl. No	Literature	Sample type	MPs Concentration	MPs Shape	MPs color	Polymer type
1	Sruthy and Ramasamy, 2017	Sediment	252.80 ± 25.76 items/m ²	Fragment, Fibre, Film,	-	PP, LDPE, HDPE, PS
2	Daniel et al., 2020	Fish, <i>Rastrelliger kanagurta</i> <i>Megalaspis cordyla</i> <i>Sardinella longiceps</i> <i>Sardinella gibbosa</i> <i>Stolephorus indicus</i> <i>Dussumieria acuta</i> <i>Thryssa dussumieri</i> <i>Sphyræna obtusata</i> <i>Anodontostoma</i>	Edible tissue- 0.07 ± 0.26 item/ fish Inedible tissue- 0.53 ± 0.77 Item/fish	Fragment, Sheet, Fibre, Foam	Red,blue, green,black, Transparent, White	PP, PE, PS, EPDM, PU, Alkyd resin, Epoxy resin
3	Devi et al., 2020	Fish	69	Fibre, Fragment,	Pink, Red, Blue,Orange,	PP, PET, PBT, Nylon 6
4	Suresh et al., 2020	Water Sediment Fish <i>Leiognathus sp.</i> <i>Ambassis ambassis</i> <i>Stelophorus sp.</i> <i>Clarias batrachus</i> <i>Cynoglossus sp.</i>	751.7 ± 452.1 1340 ± 575.22 0-5	Fibre, Fragment, Film, Foam	Red, Black, Green, Blue, White,yellow, Brown, Pink, Violet,Golde, Orange	CP, PE, PP, PS
5	Daniel et al., 2021	<i>Uroteuthis duvaucelii</i> <i>Portunus pelagicus</i> <i>Metapenaeus dobsoni</i> <i>Fenneropenaeus</i>	0.18 ± 0.48 particle/ ind 0.14 ± 0.44 particle/ ind	Fragment, Sheet Fibre	White, Blue,Black, Red, Green	
6	Nikki et al., 2021	Water Fin fish <i>Arius maculatus</i> <i>Etroplus suratensis</i> <i>Etroplus maculatus</i> Shell fish <i>Villorita sp.</i>	872 ± 573 21 ± 20 nos./ind 13 ± 5 nos. 10 ± 8 nos. Digestive part- 12 ± 15 nos./ind	Fibre, Fragment, Film, bead	Red, Black, Green, Blue, Yellow, White, Transparent	CPVC, PE, PP, PES

Table 1 - Earlier studies of MPs in water, sediment, and biota of KBW. Abbreviations: (LDPE) Low density polyethylene, (HDPE) High density polyethylene, (PP) Polypropylene, (PS) Polystyrene, (PU) Polyurethane, (EPDM) Ethylene Propylene Diene Monomer, (PET) Polyethylene terephthalate, (PBT) Polybutylene terephthalate, (CE) Cellophane, (CPVC) chlorinated polyvinyl chloride, (PES) polyester.

	Stations	Temperature (°C)	Salinity	Turbidity (NTU)	Copepod abundance (No/m3)
PSWM	1	28.5	21.01	14.1	392
	2	29	15.27	13.1	443
	3	30.3	12.15	4.8	685
	4	31.2	10.99	3.6	592
	5	32.2	4.91	14.5	181
	6	32.8	1.01	15.7	163
SWM	1	28.2	0.11	13.5	443
	2	29.1	0.1	10.9	280
	3	27.26	0.34	8.6	203
	4	30.41	0.25	8.1	217
	5	30.64	0.08	10.5	139
	6	30.92	0.08	10.3	107
NEM	1	28.42	14.4	10.2	1406
	2	29.1	7.58	9.7	994
	3	29.19	6.35	5.2	412
	4	30	4.63	7.2	320
	5	30.16	0.64	8.4	203
	6	30.3	0.12	11.3	148

Table 2 - Seasonal variation of temperature and salinity and copepod abundance in KBW.

Table 3 - Calanoid copepods with size range in the study region

	Downstream	Size (mm)	Midstream	Size (mm)	Upstream	Size (mm)
P S W M	<i>Acartia bilobata</i>	0.95- 1.10	<i>Acartia bilobata</i>	0.95- 1.10	<i>Acartia centrura</i>	1.2-1.3
	<i>A. bowmani</i>	1.2- 1.3	<i>A. bowmani</i>	1.2-1.3	<i>Acartia plumosa</i>	0.71
	<i>A. centrura</i>	1.2- 1.3	<i>A. centrura</i>	1.2-1.3	<i>Acartia spinicauda</i>	1.2
	<i>A. danae</i>	1-1.2	<i>A. erythraea</i>	1.33	<i>Acartiella gravelyi</i>	1.22
	<i>A. erythraea</i>	1.33	<i>A. plumosa</i>	0.71	<i>Acartiella keralensis</i>	1.04
	<i>A. plumosa</i>	0.71	<i>A. spinicauda</i>	1.2	<i>Allodiaptomus mirabilipes</i>	
	<i>A. southwelli</i>	0.73- 0.8	<i>Acartiella keralensis</i>	1.04	<i>Heliodiaptomus cinctus</i>	0.95- 1.40
	<i>A. spinicauda</i>	1.2	<i>Acrocalanus gracilis</i>	1.3-1.4	<i>Limnocalanus macrurus</i>	2.2-3.2
	<i>Acrocalanus gracilis</i>	1.3- 1.4	<i>Bestiolina similis</i>	0.72- 0.81	<i>Pseudodiaptomus annandalei</i>	1.18
	<i>Bestiolina similis</i>	0.72- 0.81	<i>Paracalanus parvus</i>	0.8- 1	<i>P. Binghami malayalus</i>	1.28
	<i>Labidocera acuta</i>	3-3.4	<i>Pseudodiaptomus annandalei</i>	1.18		
	<i>Paracalanus parvus</i>	0.8- 1	<i>P. serricaudatus</i>	1.29		
	<i>Pseudodiaptomus annandalei</i>	1.18				
	<i>P. serricaudatus</i>	1.29				
S W M	<i>Acartia bilobata</i>	0.95- 1.10	<i>Acartia plumosa</i>	0.71	<i>Acartia plumosa</i>	0.71
	<i>A. centrura</i>	1.2- 1.3	<i>Acartiella gravelyi</i>	1.22	<i>Acartiella gravelyi</i>	1.22
	<i>A. pacifica</i>	0.9-1	<i>Acartiella keralensis</i>	1.04	<i>Acartiella keralensis</i>	1.04
	<i>A. plumosa</i>	0.71	<i>Allodiaptomus mirabilipes</i>		<i>Allodiaptomus mirabilipes</i>	
	<i>A. southwelli</i>	0.73- 0.8	<i>Heliodiaptomus cinctus</i>	0.95- 1.40	<i>Heliodiaptomus cinctus</i>	0.95- 1.40
	<i>A. spinicauda</i>	1.2	<i>Limnocalanus macrurus</i>	2.2-3.2	<i>Limnocalanus macrurus</i>	2.2-3.2
	<i>Acartiella. gravelyi</i>	1.22	<i>Pseudodiaptomus annandalei</i>	1.18	<i>Pseudodiaptomus annandalei</i>	1.18
	<i>Acartiella keralensis</i>	1.04	<i>P. binghami malayalus</i>	1.28	<i>P. binghami malayalus</i>	1.28
	<i>Acrocalanus similis</i>	0.72- 0.81				
	<i>Allodiaptomus mirabilipes</i>					
	<i>Heliodiaptomus cinctus</i>	0.95- 1.40				
	<i>Labidocera pectinata</i>	2.1				
	<i>Limnocalanus macrurus</i>	2.2-				

	3.2				
	<i>Paracalanus aculeatus</i>	0.87			
	<i>P. crassirostris</i>	0.54			
	<i>Pseudodiaptomus annandalei</i>	1.29			
	<i>P. binghami malayalus</i>	1.18			
	<i>P. serricaudatus</i>	1.28			
N E M	<i>Acartia bilobata</i>	0.95-1.1	<i>Acartia bilobata</i>	0.95-1.1	<i>Acartia spinicauda</i> 1.2
	<i>A. bowmani</i>	1.2-1.3	<i>A. bowmani</i>	1.2-1.3	<i>A. plumosa</i> 0.71
	<i>A. centrura</i>	1.2-1.3	<i>A. centrura</i>	1.2-1.3	<i>Acartiella gravelyi</i> 1.22
	<i>A. erythraea</i>	1.3	<i>A. erythraea</i>	1.33	<i>Acartiella keralensis</i> 1.04
	<i>A. pacifica</i>	0.9-1	<i>A. plumosa</i>	0.71	<i>Allodiaptomus mirabilipes</i>
	<i>A. plumosa</i>	0.71	<i>Acartiella gravelyi</i>	1.22	<i>Heliodiaptomus cinctus</i> 0.95-1.40
	<i>A. southwelli</i>	0.73-0.8	<i>Acartiella keralensis</i>	1.04	<i>Limnocalanus macrurus</i> 2.2-3.2
	<i>Acrocalanus gracilis</i>	1.3-1	<i>Allodiaptomus mirabilipes</i>		<i>Pseudodiaptomus annandalei</i> 1.18
	<i>Paracalanus parvus</i>	0.8-1	<i>Heliodiaptomus cinctus</i>	0.95-1.40	<i>P. binghami malayalus</i> 1.28
	<i>P. crassirostris</i>	0.54	<i>Pseudodiaptomus annandalei</i>	1.18	
	<i>Pseudodiaptomus annandalei</i>	1.18	<i>P. binghami malayalus</i>	1.28	
	<i>P. serricaudatus</i>	1.29			

Table 4 - Details of MPS in calanoid copepods in the study area.

Stations	No. of MPs	Ingestion (MPs/ ind.)	Fibre %	Fibre Length (µm)	Fragmen t %	Fragment Length (µm)	Microbea d %	Microbead Length (µm)
PSWM								
1	1	0.01	100	93	-	-	-	-
2	2	0.02	50	89.5	50	93.38	-	-
3	5	0.05	60	38.2 - 90.66	20	39.03	20	11.33
4	6	0.06	50	39.63 - 51.83	17	40.04	33	14.31, 18.78
5	9	0.09	45	33.47 - 55.67	22	42.27 - 47.38	33	8.78 - 15.03
6	13	0.13	54	30.17 - 60.08	23	38.03 - 43.08	23	10.03 - 18.05
SWM								
1	0	0	-	-	-	-	-	-
2	3	0.03	67	67.02 - 108.17	-	-	33	22.07
3	4	0.04	50	53.42- 102.07	25	78.02	25	22.36
4	3	0.03	67	42.02- 93.8	-	-	33	25
5	0	0	-	-	-	-	-	-
6	2	0.02	50	35.02	50	41.54		
NEM								
1	2	0.02	50	105.39	50	43.47	-	-
2	1	0.01	-	-	-	-	100	13.45
3	3	0.03	33.3	70.32	33.3	54.69	33.3	13.05
4	4	0.04	75	65.33 - 80.39	25	42.99	-	-
5	6	0.06	50	30.27 - 53.27	17	31.17	33	9.03, 12.08
6	9	0.09	56	36.1 - 64	11	30.34	33	8.09 - 18.09

Table 5 - Details of earlier studies on MPs in copepod world over.

Author	Location	Environment	Copepoda / Species / Order	Concentration (No. / Ind.)	Morphology	Size (µm)	Color	Polymer
Desforges et al., 2015	Northeast Pacific Ocean	Sea	<i>Neocalanus cristatus</i>	0.03 ± 0.01	Fibre Fragment	951 ± 269 196 ± 29	Black, Blue, Red	-
Sun et al., 2017	Northern part of south china Sea	Sea	Copepoda	0.05	Fibre, Particles, Others	150	-	Polyester
Sun et al., 2018a	East China Sea	Sea	Copepoda	0.12 ± 0.15	Fibre Fragment Pellet	295.2 ± 348.6 68 ± 42 22 ± 9	-	Organic oxidation polymers
Sun et al., 2018b	Yellow sea	Sea	Copepoda	0.09	Fibre, Fragment, Pellet	154.62 ± 152.90	-	Organic oxidation polymers
Kosore et al., 2018	Kenya coastal	Sea	Copepoda	0.33	Film, Fragment	-	Black, Red, Brown, Blue, Green	Low density polyethylene
Zheng et al., 2020	Bohai Sea, China	Sea	Copepoda	0.027	Fibre, Fragment	-	Blue, Black, Red, Brown, White, Gray, Green	Cellophane, Polyethylene terephthalate, Organic oxidation polymers
Goswami et al., 2020	Port Blair Bay, Andaman Islands	Sea	Copepoda	0.028 ± 0.023	Fragment, Fibre, Pellet	-	-	Ionomer surlyn, Acrylic, Nylon
Amin et al., 2020	Terrengannu coastal waters southern south china Sea	Sea	Calanoida Cyclopoida	0.13 0.007	Fibre Fragment Fragment	313 56.3 23.5	-	Polyamide
Buathong et al., 2020	Upper gulf of Thailand	Sea	Calanoida	0.45 ± 0.04	Fibre	458.6 ± 311.3	Blue, Red,	Polyethylene

			Cyclopoida	0.50 ± 0.03		517.9 ± 319.9	Brown, Black	terephthalate, Polyurethane, Rayon
			Harpacticoida	-		-		
Aytan et al., 2020	Southeastern Black Sea	Sea	<i>Acartia clausi</i>	0.002	Fragment	121 ± 128	Black, Red, Blue	-
					Film	38 ± 15		
			<i>Calanus euxinus</i>	0.004	Fragment	66 ± 43		
Zheng et al., 2021	Jiaozhou Bay	Sea	Copepoda	0.21 ± 0.10	Fibre	473 ± 384	-	Polyester, Cellophane
					Fragment	220 ± 72		
Aytan et al., 2021	Black Sea	Sea	<i>Acartia clausi</i>	0.008 ± 0.006	Fragment, Film, Fibre	100 ± 153	Black, Blue, Red	Polyethylene terephthalate
			<i>Calanus euxinus</i>	0.024 ± 0.020		62 ± 56		
Rashid et al., 2021	Eastern arabian sea	Sea	Copepoda	0.03 ± 0.01	Fibre, Fragment, Film, Pellet	195 ± 42	Blue, Black, Red, Green, Transparent	Polypropylene
Taha et al., 2021	Terengganu estuary and offshore, Malaysia	Sea	Calanoida	0.03 ± 0.01	Fibre, Fragment	-	-	Nylon, Polyethylene, Polypropylene
			Cyclopoida	0.02 ± 0.02				
		Estuary	Calanoida	0.02 ± 0.02				
			Cyclopoida	-				
			Harpacticoida	-				