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# Seasonal variation in benthic meiofaunal diversity and assemblage in Punnakayal estuary, Tuticorin, India; Multivariate approach

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### **ABSTRACT**

In the present study, benthic meiofaunal diversity and distribution in Punnakayal estuary was studied and a total of 35 meiobenthic species belonging to four meiofaunal taxa foraminiferans, nematodes, ostracods and harpacticoids were recorded with maximum density of meiofauna (248Nos/10cm3) in St-3. Among the four meio-faunal taxa, nematodes topped the list with 14 species followed by foraminiferans (10 species), ostracods (8 species) and harpacticoids (3 species). Seasonally, the maximum number of meiofaunal species (24species) was recorded at St-3 during postmonsoon and minimum (13 species) was recorded at St-5 during monsoon. CCA and BIO-ENV (Biota-Environmental matching) analysis showed that the environmental parameters such as DO, W. pH, salinity, silt, clay and TOC manifested as best match ( $\rho\omega$  = 0.931) in determining meiofaunal distribution in the surveyed stations. The maximum meiofauna diversity (3.396) and evenness (0.783) was recorded at St-3 and similarly the maximum species richness was recorded (6.539) at St-5. The results of present study helps to develop an understanding on the meiofaunal distribution based on physico-chemical parameters, which will form a reliable tool in bio-monitoring studies.

KEYWORDS: Meiofauna, density, diversity, Punnakayal estuary, India

### 1. INTRODUCTION

Estuaries are the partially enclosed coastal body of water with one or more rivers or streams flowing into it and with a free connection to the open sea. Estuaries are among the most productive ecosystems of the world (Lindeboom, 2002). Estuaries are exclusive and unique hot spots of biodiversity, which supports a plethora of organisms. They act as critical reproductive and nursery

ground for a variety of organisms. The linkage and gateway function of estuaries between marine and fresh water environment is an essential feature in the life cycle of several invertebrates (Allen *et al.*, 2012). This integrative processes of tying together terrestrial, fresh water and marine biomes, weave a web of complexity far greater than that of their three contributor systems. The benthic fauna are the critical component of shallow

water estuarine and coastal marine ecosystems (Paolo, 2003). They regulate the physical, chemical, and biological environment of the estuary and link the sediment to aquatic food web, through their burrowing and feeding activities (Brigolin et al., 2011; Hochard et al., 2012; Brady et al., 2013). The benthic system comprehends a highly diverse community, composed of bacteria, micro- meio- and macrobenthos, with the classification of benthic organisms generally relying on the organism size, as Macrofauna (> 0.5mm), Meiofauna (0.5 to 0.063mm) and Microfauna (< 0.063mm). Meiofauna, or more generally, the interstitial benthic invertebrates are distinguished from macro benthos by their smaller sizes and shares tremendous amount of total benthic biomass in marine habitats (Chakraborty and Datta, 2018).

Benthic meiofauna is an important group of organisms in the estuarine ecosystem feeding on microalgae and bacteria. They play an important role bio- mineralization (Moghadasi et al., 2009; Nari Mesa et al., 2011). Compared to macrofauna, meiofauna is highly useful in environmental impact assessment and ecosystem health monitoring in view of its higher species richness, short life-cycles (3-5 generation per year) and lack of larval stages (Ansari et al., 2012). This morphologically and taxonomically important group comprises of diverse organism representing wide range of invertebrate taxa; nematodes and harpacticoid copepods other groups include turbellarians, ostracods, gastrotichs, tardigrades, rotifers, polychaetes, oligochaetes, gastropods and bivalves (Urban-Malinga, 2013). Most of the studies on the meiofaunal diversity in the Indian subcontinent have been done in the continental shelf (Harkantra et al., 1980; Sajan and Damodran, 2007; Sajan et al., 2010) and shallow coastal waters (Timm, 1961, 1967; Rao and Ganapati, 1968; Ansari, 1978; Ansari et al., 1980; Rodrigues et al., 1982), very few such studies has been carried out in the estuarine waters and therefore in this study an attempt is made to document the diversity and assemblage of benthic meiofauna from Punnakayal estuary, Tuticorin with reference to seasonal variation in environmental parameters.

### 2. MATERIALS AND METHODS

### Study area

In the present study, the seasonally sampling was carried out in Punnakayal estuary waters (08°38'44.04"N and 78°07'16.61"E) for a period of one year from July 2021 (Pre monsoon) to June 2020 (Summer). Five sampling sites were selected based on the depth (Table 1, Fig. 1). The monthly data were amalgamated to seasons and the results are presented seasonally. The details of sampling stations are given below:



Fig. 1. Map showing the sampling stations in Punnakayal estuary waters

Table 1. Geographical locations of sampling stations in Punnakayal estuary waters

Station code	<u>Latitude</u>	Longitude	Station details
St-1	8°38'44.04"N	78°06'57.35"E	Nearby Agriculture Land
St-2	8°38'33.09"N	78°07'08.02"E	Nearby Waste Land
St-3	8°38'28.22"N	78°07'27.08"E	Nearby Mangrove zone
St-4	8°38'15.94"N	78°07'31.03"E	Fixed near 500 m Coastal Mouth
St-5	8°38'15.46"N	78°07'16.61"E	Fixed near fishing landing Centre

### Water and sediment analysis

Measurements of temperature (hand-held mercury thermometer), salinity (Refractometer, ATAGO, Japan) and pH (pH pen, model LI-120, Eutech Instrument, Singapore) were recorded during each sampling event. Dissolved oxygen (DO) was estimated by using Winkler's method (Strickland & Parsons, 1972). Undisturbed surface-sediment subsamples were shade dried for estimation of Petroleum Hydrocarbon (PHC), Total Organic Carbon (TOC) and sediment texture. The sediment grain-size analysis (i.e., sediment texture) was

done by following pipette method of Krumbein & Pettijohn (1938). TOC estimation was done by following Topping (1972) and the level of heavy metals was detected by using Inductively Coupled Plasma Mass Spectrophotometer (AGILENT -7700x ICP-MS). Total petroleum hydrocarbon analysis in sediment sample was done by using the methods of Laboratory Analytical Work Instruction, 2011.

### Benthic meiofauna analyses

For faunal analysis, a total of six surface three replicate sediment samples were collected using a Van Veen grab with a sampling area of 0.1m<sup>2</sup>; depth ranging between 8.0 and 12.0 m (Fig. 1 & Table 1), sub-sampling the top layer of each grab (~1 cm thick) and samples were then stored in cold box in sealed plastic bags. Sediment subsamples (~100 g) for meiofauna analysis were placed in labeled plastic bags, immediately fixed in 4% buffered formalin in distilled water, and brought to the laboratory. The sediments were washed with tap water through a set of 0.5 mm and 0.063 mm sieves. The sediment retained on the 0.063 mm sieve was decanted to extract meiofauna following the methodology of Higgins & Thiel (1988). Sorting of metazoan meiofauna (foraminiferans, nematode, harpacticoids, ostracods) from sediment was done by flotation and decantation using a sieve with 0.040 mm mesh size; the efficiency of this technique has been reported as 95% by various researchers (Somerfield & Warwick, 1996; Danovaro et al., 2004; Giere, 2009). The organisms retained on the sieve were placed into Petri dishes for sorting and preserved in 70% ethyl alcohol with 5% glycerol (Tolhurst et al., 2010). A few drops of Rose Bengal (1 g/l) were also added to this solution to facilitate the counting process. Subsequently, the sorted meiobenthic organisms were counted and identified to species level under a stereomicroscope (EISCO Stereo Binocular Microscope) by consulting the standard works of Loeblich and Tappan (1994), Coccioni et al. (2009), Frontalini et al. (2010), Mohan et al. (2013), Loeblich and Tappan (2015), Brunović et al. (2019), Ballesteros-Prada (2019) and Hayward et al. (2020) for foraminifera; Chitwood (1958), Lambshead (2004), De Ley et al. (2005), Poinar (2008), Vovlas et al. (2011) and Ahmed et al. (2015) for nematodes; Brouwers et al. (2000), Tanaka (2008) and Yasuhara et al. (2014) for ostracods; and Huys & Boxshall (1991), Wells (2007) and Yeom & Lee (2020) for harpacticoids. The numerical

abundance of the meiofauna was expressed in individuals per 10 cm<sup>3</sup> (Fernando *et al.*, 1983).

### Statistical analysis

In order to correlate the seasonal variation in environmental parameters with meiofaunal distribution and assemblage the data were statistically analysed using univariate and multivariate methods available in the statistical software PRIMER (Ver. 7.0) (Clarke et al., 2016). The diversity index (H') (Shannon & Wiener, 1949), richness index (d) (Margalef, 1958), evenness (J') (Pielou, 1966), and dominance (D) (Simpson, 1949) were calculated using benthic meifaunal species abundance. The statistical package 'R' (v. 3.4.4; Oksanen et al., 2017) was used for Principal component analysis (PCA) to visualize correlation between the physico-chemical parameters and sampling stations and similarly, Canonical Correspondence Analysis (CCA) was also done to relate the abundance of meiofaunal taxa with linear representations of environmental variables.

### 3. RESULTS

## Physico-chemical characteristics of water and sediment

The values of physico-chemical parameters of water and sediment are summarized in Table. 2. Temperature ranged from 25.0 to 31.5°C and the minimum was recorded at St-2 during monsoon and maximum was at St-5 during summer. Salinity fluctuated between 8.0 and 30.5 ppt, with the minimum was recorded at St-1 during monsoon and maximum was at St-4 during summer. Water pH varied from 8.3 at St-4 during summer and 7.6 at St-2 during monsoon. Dissolved Oxygen (DO) ranged from 3.85 mg/l at St-5 during summer and 5.47 mg/l at St-2 during monsoon. In the sediments, the TOC content ranged from 6.32 to 10.64 mgC/g and the maximum was recorded at St-2 during monsoon and minimum was at St-4 during summer. Sand content ranged between 10.34 and 83.66%, with maximum value was recorded at St-4 during summer and minimum at St-1 during monsoon; silt content varied from 15.40 to 21.60% with maximum at St-3 during monsoon and minimum at St-5 in summer and the clay content in the sediment fluctuated between 18.72 and 84.18% with maximum (St-1) in monsoon and minimum (St-4) in summer.

The Lead content varied from 0.91 to 1.32 mg/kg with the maximum at St-5 during summer and

minimum at (St-1) during monsoon. Copper values ranged between 0.97 and 3.11 mg/kg with the maximum was recorded during summer at (St-4) and minimum was during premonsoon at (St-2). Cadmium varied from 1.16 to 2.64 mg/kg with the maximum level was

recorded at (St-5) during summer and minimum at (St-1) during monsoon. Chromium level varied from 0.57 to 2.18 mg/kg and the maximum level was recorded at (St-5) during summer and minimum at (St-2) during monsoon (Table 2).

Table 2. Physico-chemical characteristics (mean and SD) recorded in various sampling stations of Punnakayal estuary waters.

Stations ID	St-1	St-2	St-3	St-4	St-5
Depth	$2.5 \pm 0.45$	$3.0 \pm 0.72$	$2.5 \pm 0.48$	$3.5 \pm 0.80$	$3.1 \pm 0.61$
Temp. (°C)	$27.0 \pm 2.18$	$25.0 \pm 1.54$	$28.0 \pm 1.73$	$28.5 \pm 1.26$	$31.5 \pm 1.84$
Salinity (ppt)	$8.0 \pm 1.32$	$15.5 \pm 1.36$	$18.5 \pm 1.41$	$26.0 \pm 1.59$	$30.5 \pm 1.63$
Water pH	$7.6 \pm 0.26$	$7.8 \pm 0.19$	$8.0 \pm 0.27$	$8.3 \pm 0.30$	$8.2 \pm 0.49$
DO (mg/l)	$4.21 \pm 0.85$	$5.47 \pm 1.63$	$4.34 \pm 1.50$	$4.58 \pm 1.24$	$3.85 \pm 1.07$
TOC (mgC/g)	10.39 ± 1.66	$10.64 \pm 1.45$	$8.51 \pm 1.04$	$6.32 \pm 0.75$	$8.49 \pm 0.83$
Sand (%)	$10.34 \pm 1.94$	$12.85 \pm 2.01$	22.64 ± 1.75	83.66 ± 1.07	25.43 ± 1.65
Clay (%)	$84.18 \pm 2.75$	76.51 ± 2.53	$53.10 \pm 2.07$	25.68 ± 2.13	18.72 ± 2.55
Silt (%)	$13.79 \pm 0.60$	12.44 ± 1.08	$21.60 \pm 1.35$	$13.65 \pm 1.52$	$15.40 \pm 0.92$
Pd (mg/kg-1)	$0.91 \pm 0.13$	$0.95 \pm 0.09$	$0.94 \pm 0.16$	$1.14 \pm 0.18$	$1.32 \pm 0.24$
Cu (mg/kg-1)	$0.99 \pm 0.26$	$0.97 \pm 0.16$	$1.04 \pm 0.22$	$3.11 \pm 0.31$	$2.93 \pm 0.57$
Cd (mg/kg-1)	$1.16 \pm 0.55$	$1.42 \pm 0.39$	$1.38 \pm 0.37$	$1.85 \pm 0.62$	$2.64 \pm 0.35$
Cr (mg/kg-1)	$0.84 \pm 0.21$	$0.57 \pm 0.07$	$0.61 \pm 0.25$	$1.47 \pm 0.51$	$2.18 \pm 0.41$

(Footnote: Temp - Temperature; DO - Dissolved Oxygen; TOC - Total Organic Carbon; Fe - Iron; Pd - Lead; Cu - Copper; Cd - Cadmium; Cr - Chromium)

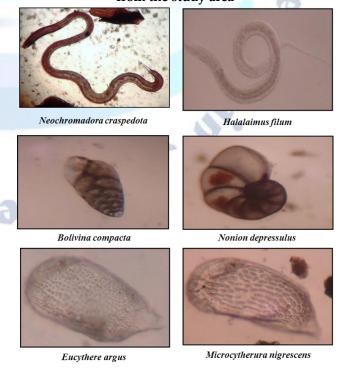
### **Benthic Meiofaunal**

In the present study, as many as 35 species belonging to four groups of benthic meiofauna namely nematodes, foraminiferan, ostracods and harpacticoids were recorded in various stations in Punnakayal estuary waters. Among them, nematodes topped the list with 14 species; foraminiferans were found to be the next dominant group with 10 species; ostracods came next with 8 species and harpacticoida came last with 3 species only.

Among the nematodes, *Desmodora cambelli*, *Halalaimus filum* and *Astomonema jenneri* were found to be the common species in the surveyed stations. With respect to foraminiferans, *Ammonia beccarii*, *A. tepida*, *Bolivina limbata*, *Elphidium texanum*, *Rosalina globularis* and *Trochammina adaperta* were found commonly in various stations. The ostracods species such as *Basslerites liebaui*, *Bairdoppilata scaura*, *Keijella reticulate* and Harpacticoids, *Laophonte thoracica*, *Paramesochra dubia* and *Macrosetella gracilis* were found to be common in the surveyed stations. The maximum abundance was recorded at station St-3 with 248 individuals/10 cm³ during postmonsoon and the minimum was at St-5 with 86 individuals/10 cm³ during monsoon. Seasonally, the

maximum number of meiofaunal species (24 species) was recorded at St-3 during postmonsoon and minimum (13 species) was recorded at St-5 during monsoon (Plat 1).

Plate 1. Dominant benthic meiofauna species recorded from the study area



### Percentage contribution

The results of percentage composition of meiofauna revealed that nematodes constituted the maximum with 45% of the total meiobenthic organisms. Foraminiferans, ostracods and harpacticoids contributed with 28%, 18% and 9% respectively to the total meiobenthic samples collected from Punnakayal estuary waters (Fig. 2).

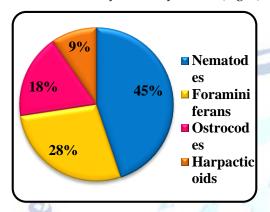


Fig. 2. Percentage contribution of meiofaunal groups recorded in various sampling stations of the Punnakayal estuary waters

### **Diversity Indices**

Shannon diversity (H') index lowest value was recorded (2.175) at St-5 during monsoon and highest (3.396) was at St-3 during postmonsoon. Margalef species richness (d) lowest value was (3.817) at St-1 during monsoon and highest (6.539) at St-5 during summer. Pielou's species evenness (J') varied between 0.429 and 0.783 with the lowest value in St-4 during monsoon and the highest in St-2 during summer. Simpson dominance index varied from 0.682 to 0.743 with lowest in St-5 during monsoon and the highest in St-2 during postmonsoon (Table 4).

Table 9. Diversity indices Shannon diversity (H'); Margalef richness (d), Pielou's evenness (J') and Simpson dominance (D) calculated for Meiobenthos in Punnakayal estuary waters

Stations	Shannon diversity (H')	Margale <mark>f rich</mark> ness (d)	Pielou's evenness (J')	Simpson dominance (D)
St-1	3. <mark>021</mark>	3.817	0.607	0.684
St-2	2.815	4.362	0.783	0.743
St-3	3.396	6.317	0.747	0.669
St-4	3.302	4.726	0.429	0.653
St-5	2.175	6.539	0.536	0.682

### PCA and CCA

The principal component analysis was performed using physico-chemical parameters to set a well-defined distinction between the stations. The PCA plot revealed that St-2, St-4 and St-5 showed high correlation with parameters such as DO, salinity, water pH, sand, evenness (J) and diversity (H'); while St-1 and St-3 negatively correlated with other parameters such as water silt, PHC and heavy metals (Fig. 3). Similarly, CCA analysis was done to find out the correlation environmental parameters and species abundance, which revealed that the species such as Ammonia beccarii, A. tepida, Bolivina limbata, Elphidium texanum, Quinqueloculina granulocostata, Rosalina globularis, Spirillina lateseptata, Spiroloculina excavate, Desmodora cambelli, Halalaimus filum, Astomonema jenneri, Basslerites liebaui, Neocytherideis senescens, Keijella reticulata, Paijenborchella cymbula, Macrosetella gracilis got positively correlated with temperature, W. pH, salinity,

dissolved oxygen, sand, clay, and total organic carbon at St-2, St-4 and St-5. It was also evident that species such as Globorotalia hirsute, Lagena lacunata, Neouvigerina hispida, Nonion grateloupi, Orbulina universa, Pararotalia ozawai, Sorites Triloculina tricarinata, marginalis, Trochammina adaperta Oxystomina clavicauda, Enoplolaimus abnormis, Oxystomina clavicauda, Enoplolaimus abnormis, Bairdoppilata scaura, Stenocypris major, Laophonte thoracica, Paramesochra dubia were negatively related to the silt, petroleum hydrocarbon and heavy metals at St-1 and St-3 (Fig. 4).

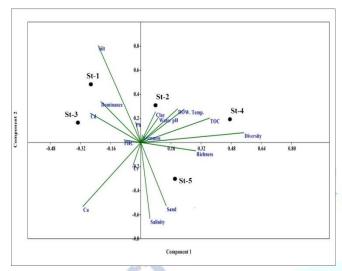


Fig. 3. Principal Component Analysis plot drawn for environmental parameters and meiofaunal diversity in Punnakayal estuary waters

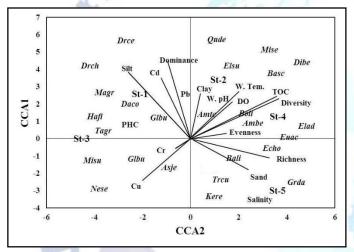


Fig. 4. CCA plot showing inter-relationship between meiofauna species against environmental variables in Punnakayal estuary waters

#### 4. DISCUSSION

Worldwide urbanization and industrialization led to widespread contamination of coastal environments. As observed above, the distributions, abundance, diversity, and composition of benthic meiofaunal assemblages in estuarine environment is controlled largely by a combination of various physico-chemical parameters (temperature, salinity, currents, substrate, sediment type and vegetation cover), food resources and biotic interactions (Ellis *et al.*, 2018). In the present study, the high water temperature, salinity and pH values were observed in stations St-4 and St-5, which might be due to proximity to the marine backwater zone and lower values were recorded in stations near to freshwater zone St-1, St-2 and St-3 receiving freshwater influx.

Among the seasons, the maximum values were recorded during summer which might be due to low rain fall and the rise in atmospheric temperature (Al-Dubai *et al.*, 2017, Kucharska *et al.*, 2019).

The dissolved oxygen (DO) was found maximum in stations near to freshwater zone St-1 during monsoon season and minimum at stations near to estuarine mouth St-4 and St-5 during summer season. The relatively minimum DO values observed in the summer/pre monsoon are attributed to fluctuations in temperature and salinity, which in turn affect the dissolution of oxygen. Similar observation was made earlier by Amao et al. (2019) in Iranian coast and Nagendra and Reddy (2019) from Uppanar Estuary, India. TOC content varied from 6.32mgC/g to 10.74mgC/g and the maximum was recorded at freshwater stations St-1 during monsoon and minimum at marine backwater station St-5 during summer. The maximum TOC in coastal stations might be attributed to clayey nature of sediment (sandy loams, sandy clay, clay loams and clays). Organic matter, as a food source, play<mark>s a</mark> key r<mark>ole in determining</mark> the meiofaunal dist<mark>ribut</mark>ions (Rombouts et al., 20<mark>13; M</mark>artins et al., 2015). Similarly, Frontalini et al. (2014) also opined that the sediment characteristics and the total organic carbon (TOC) contents influence the distribution of meiofauna in Lake Varano, Southern Italy.

Bottom sediments cannot be considered as a sink of pollutants and the metal mobilization in the sediment environment may take place, depending on the physico-chemical changes. In the present study, heavy metal concentration also varied significantly in both the nearshore and offshore stations. The level of Cadmium (Cd), Lead (Pd) and Chromium (Cr) accumulation was found maximum at fishing landing center stations (St-5 and St-4) and minimum at freshwater stations (St-1, St-2 and St-3). The higher concentration of metals in fishing landing center stations could be attributed to the heavy rainfall and subsequent river runoff and agricultural wastes, which include residue of heavy metal containing pesticides. Ananthan et al. (2006); Karthikeyan et al. (2007); Chitrarasu et al. (2013) also reported similar trend of heavy metal distribution in Ennore estuary, India and Jeshma et al. (2016) in Karaikal coastal waters. The values recorded in the present study are comparable to the reports made by Kesavan and Ravi (2013); Gandhi and Nathan (2014) and Nagendra and Reddy (2019) from Uppanar estuary, India.

A total of 35 meiofaunal species belonging to four groups of benthic meiofauna namely nematodes, foraminiferan, ostracods and harpacticoids were recorded in various stations in Punnakayal estuary waters. Among them, nematodes topped the list with 14 species; foraminiferans were found to be the next dominant group with 10 species; ostracods came next with 8 species and harpacticoida came last with 3 species only. Among the nematodes, Desmodora cambelli, Halalaimus filum and Astomonema jenneri were found to be the common species in the surveyed stations. With respect to foraminiferans, Ammonia beccarii, A. tepida, Bolivina limbata, Elphidium texanum, Rosalina globularis and Trochammina adaperta were found commonly in various stations. The ostracods species such as Basslerites liebaui, Bairdoppilata scaura, Keijella reticulate and Harpacticoids, Laophonte thoracica, Paramesochra dubia and Macrosetella gracilis were found to be common in the surveyed stations. The lower diversity and density of meiofauna was recorded in fishing landing center stations, which might be due to the freshwater influx and shallow depth, leading to unfavorable environment for meiofaunal population. Similar range of meio-faunal density was also reported earlier by Pusceddu et al. (2014) in East coast of the Yucatan peninsula (Mexico); Khalil (2019) in Red Sea coast, Sudan.

Species diversity can be an expression of the environmental stress on benthic assemblages, with higher diversity in more stable environments. Species diversity and evenness value was found minimum in estuary stations during monsoon and maximum in marine backwater stations during summer season, which might be due to the influence of freshwater influx, temperature and low salinity as reported by Xuan Quang et al. (2013). Similar range of meiofaunal diversity values were also reported by Frontalini et al. (2018) from Conero coast, Adriatic. Species richness and dominance showed minimum value at offshore stations during pre-monsoon and higher value in nearshore stations in summer. Similar trend was also observed by Chen and Lin (2017) from Dongsha Lagoon, China.

### 5. Conclusion

The present study provided base line information about the diversity and distribution of meiofauna in Punnakayal estuary. The present findings contribute additional knowledge on the influence of seasonal variation on the meiofaunal assemblage, since there is only a very few studies on this aspects in Indian coastal waters. Analysis of data undertaken with conventional tools like univariate and multivariate methods clearly revealed the healthy nature of the coasts and species estimation showed that the sample size of present study is quite adequate for the efforts taken to document all the meiofaunal species occurring in the surveyed estuary. Moreover, this study also emphasized that temperature, DO, sediment texture, salinity and pH are the most important factors in determining the distribution of meiofauna in Punnakayal estuary.

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### Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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