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Species Diversity and Community Assemblage of Planktonic Rotifers from Vembanad Estuary-Kerala, India

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ABSTRACT

Among the zooplankton, Rotifera comprise an integral part of the food chain and are important link between nanoplankton and carnivorous zooplankton. The diversity of rotifers is indicative of the ecological factors of the water body as they respond more quickly to environmental changes and appear as sensitive indicators of changes of water quality. In this context, species diversity of planktonic rotifers and its community assemblage in Vembanad backwater (Ramsar site) has been carried out from March 2012 to February 2013 from ten selected stations on North and South of the Thanneermukkom barrage. Abiotic and biotic parameters were determined and correlated with rotifer abundance to gain information about the forces that assembles the rotifer community in this dynamic environment. Maximum abundance was observed during monsoon (av. 8934 individual m^{-3}) followed by premonsoon (av.5549 individual m^{-3}) and postmonsoon (av. 4011 individual m^{-3}). During the present study 26 species of the rotifers belonging to 10 families were observed, with maximum abundance of *Brachionus* sp. (60%). Predominant species were *Brachionus rotundiformis* (16.4%), *B. calyciflorus* (6.6%), *B. urceolaris* (5.5%), *B. plicatilis* (4.05%), *Keratella tropica* (3.2%) and *Asplanchna* sp. (2.2%). Most of the dominant species observed were eurytopic freshwater forms that became less abundant towards downstream (station 8, 9 and 10) with respect to the salinity gradient prevailing in the estuary. Highest Shannon diversity index (4.51) was observed in station 3 (Rani) and lowest (2.73) in station 10 (Aroor). Multivariate Bio-Env (BEST) analysis showed that temperature, conductivity, salinity and phosphate best determined the abundance and distribution of rotifers in the Vembanad estuary. As the trophic status and rotifer assemblages of an ecosystem are very much related, a comprehensive study on the species diversity and community assemblage of rotifers in Vembanad estuary can furnish a valuable integrative index for further ecological assessment and monitoring of estuarine ecosystems.

Key words: Rotifers, ecology, species diversity, Vembanad, estuary

INTRODUCTION

Rotifers are an important component of planktonic communities and an integral link of aquatic foodwebs. Among the zooplankton, rotifers respond more quickly to the environmental changes and are used as bio-indicators of water quality (Gannon and Stemberger, 1978; Sladecek, 1983; Berzins and Pejler, 1989; Sheeba and Ramanujan, 2005). As, they are the first metazooplankters by grazing on the phytoplankton, qualitative and quantitative studies of rotiferan group of zooplankton diversity are of great significance. Their occurrence is affected by the complex interaction of various physical, chemical, geographical, biological and ecological parameters. All these factors play an individual role in the formation of rotifer assemblages and their seasonal occurrence, but the ultimate effect is produced due to interplay and interaction of all these factors (Hulyal and Kaliwal, 2008). They are ubiquitous, occurring in all types of fresh and brackish water habitats. Nearly, 95% of species are reported from freshwater habitats and remaining 5% are from marine environment.

Estuarine zooplankton studies which took rotifers into consideration were conducted in the Hudson (Pace *et al*., 1992; Vaque *et al*., 1992), Sacramento Rivers (Orsi and Mecum, 1986), Belgium coastal areas (De Ridder and Verheye, 1980) and in San Francisco Bay (Ambler *et al*., 1985) etc. Taxonomic studies on Indian rotifers was commenced by Anderson (1889) and later by Ahlstrom (1940), Pasha (1961), Arora (1966), Naidu (1967), Nair and Nayar (1971), Vasisht and Battish (1971). The available information on the Indian rotifer fauna was reviewed by Sharma and Michael (1980) and confirmed presence of 241 species of rotifers from the Indian subcontinent. Even though, the Indian rotifer fauna is relatively well-studied (Segers *et al*., 1994), studies on its distribution and ecology are limited and are mostly related to the ecosystems of northern parts of the country. First report on rotifers of Kerala was given by Nayar (1968). Availability and abundance of rotifers in certain brackish water regions of southern Kerala was documented by Nandan (1991), Harikrishnan (1993) and Anuradha (1996) while, studying the general plankton communities in these areas. Anitha (2003) investigated the systematics of rotifers with special emphasis on the family Brachionidae of Veli-Aakulam and Poonthura estuaries. Even though rotifers of Cochin backwaters have been studied by several authors (Gopakumar, 1998; Varghese and Krishnan, 2009), a survey of the rotifer group of zooplankton and analysis in Vembanad estuary is scarce. Hence, in the present study, a survey of the rotifers, its species diversity, spatio-temporal distribution within the estuary and community assemblage along with prevailing environmental conditions is envisaged.

MATERIALS AND METHODS

Study area: The Vembanad estuarine system, a Ramsar site on these southwest coast of India (lat 9°30'069"-9°53'519"N and long 76°21'268"-76°18'139"E) extending from Punnamada in South to Aroor in North was selected for the study. Monthly field sampling was conducted in ten different stations, with six stations on southern side of the barrage (upstream) and 4 on Northern side of barrage (downstream) which are ecologically different and with varied environmental parameters. The study stations where, St. 1, Punnamada, St. 2, Pallathuruthy, St. 3, Rani, St. 4, Aryad, St. 5, Pathiramanal, St. 6, Thaneermukkom South, St. 7, Thaneermukkom North, St. 8, Varanad, St.9, Perumbalam and St. 10, Aroor during the period from February 2012 to January 2013. The map showing the study stations are given in Fig. 1.

Rotifers: The plankton samples were taken from each station by filtering 5 L of water through conical plankton net made up of bolting silk having a mesh size of 40 μm. The filtered plankton samples were preserved using 4% formaldehyde. Rotifers were identified with standard keys (Ward and Whipple, 1958; Sharma, 1983). A Sedgewick-Rafter chamber was used to count the rotifers at 40-100x magnification with a light microscope (Leica DM 500). Rotifer community could thus be described from both a qualitative and quantitave perspective using data expressed in terms of abundance (individual m^{-3}).

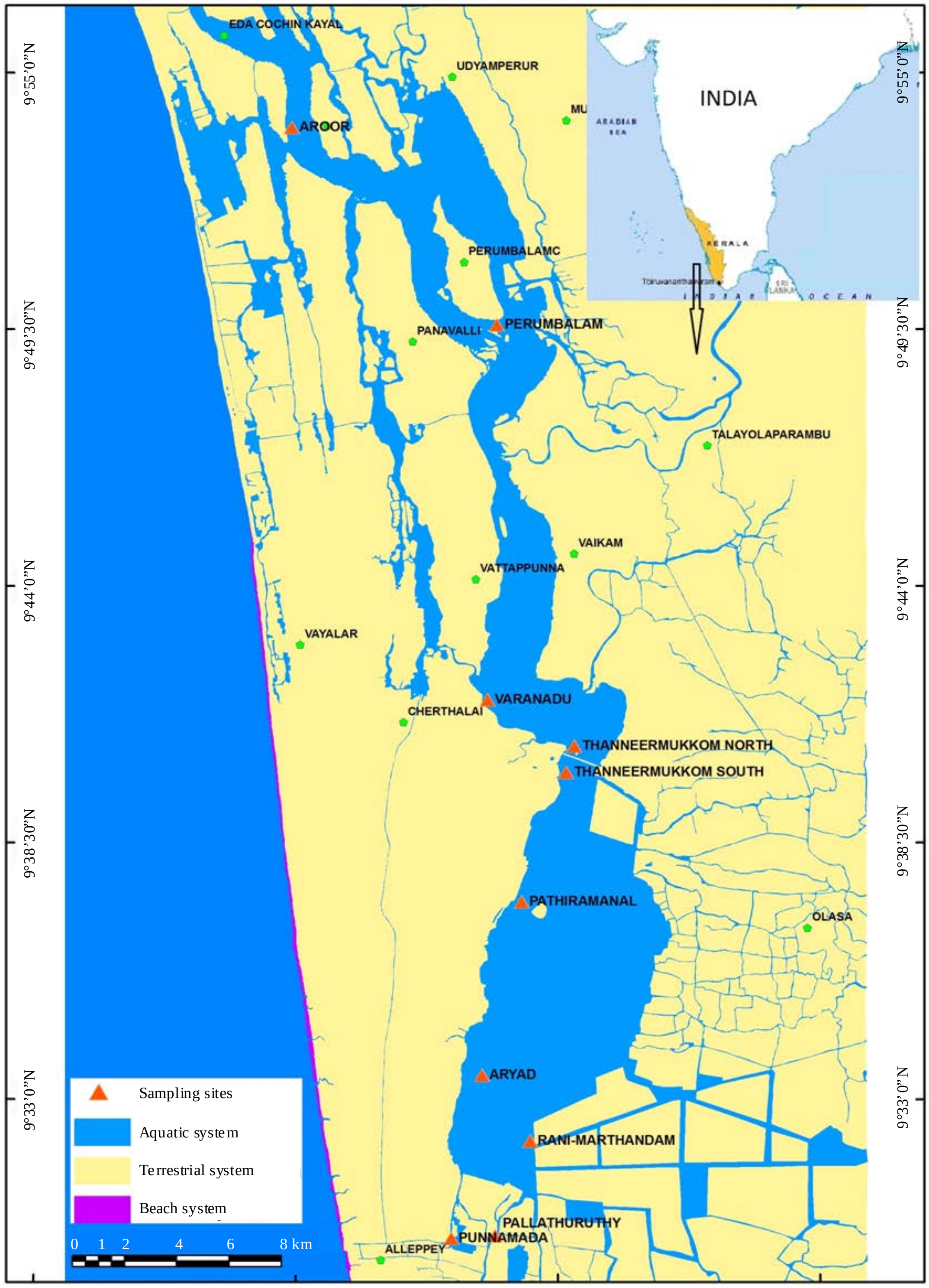

Fig. 1: Map showing study stations in Vembanad estuary

Water sample collection and analysis: Surface water samples were collected using Niskin water sampler (Hydrobios 5 L) and were kept in the icebox and brought to the laboratory. Winkler's

Table 1: Species check list of rotifers from Vembanad estuary during 2012-2013

Orders	Family	Genus	Species
Ploimida	Asplanchnidae	*Asplanchna*	*A. herricki* Gosse
			A. priodonta Gosse
Ploimida	Brachionidae	*Brachionus*	*B. plicatilis* Muller
			B. urceolaris Muller
			B. calyciflorus Pallas
			B. diversicornis Daday
			B. falcatus Zacharias
			B. angularis Gosse
			B. forificula Nair and Nayar
			B. quadridentatus Herman
			B. caudatus Barrois and Daday
			B. rotundiformis Tschugunoff
			B. havanaensis Rousselet
Ploimida	Brachionidae	*Keratella*	*K. tropica* Apstein
			K. tropica f. aspina Fadeew
			K. tropica f. assymetrica Barrois and Daday
			K. cochlearis Gosse
Ploimida	Brachionidae	*Platiyas*	*P.* quadricornis Ehrenberg
	Notommatidae	*Cephalodella*	*Cephalodella* sp.
Ploimida	Synchaetidae	*Polyarthra*	*P. vulgaris* Carlin
Ploimida	Trichocercidae	*Trichocerca*	*T. cylindrica* Imhof
Flosculariacea	Hexarthridae	*Hexarthra*	*H. intermedia* Wizniewski
Flosculariacea	Filiniidae	*Filinia*	*F. terminalis* Plate
			F. longiseta Ehrenberg
Flosculariacea	Testudinellidae	*Testudinella*	*T. patina* Herman

titrimetric method (Strickland and Parsons, 1972) was followed for the estimation of Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD). The pH, temperature and salinity was measured by Systronics water analyser (model No.317). Water samples were analysed for dissolved inorganic micro nutrients-nitrate-nitrogen (Zhang and Fischer, 2006), phosphate-phosphorus and silicate silicon (APHA., 2005). For chlorophyll 'a' analysis, water samples were filtered immediately after collection and estimated based on standard procedure (APHA., 2005).

Data analysis: Two-way Analysis of Variance (ANOVA) was performed for water quality parameters (temperature, pH, salinity, dissolve oxygen and nutrients) to investigate existence of any statistical significance among the stations and seasons using SPSS version 20. Pearson's correlation test was also performed to evaluate the relationships between the rotifer species with various observed environmental parameters that might be influencing their population. Data was further subjected to hierarchical cluster and multidimensional scaling (MDS) analyses for out the similarity ranking of rotifer composition between different stations. The BEST (Bio-Env+Stepwise) analysis was also computed to assess the correlation of water quality parameters with rotifers. Diversity indices (Table 1) such as Shannon-Wiener diversity (H'), Margalefs Richness index (d) and Pielou's eveness index (J') were computed using PRIMER Vs. 6.0 (Clarke and Gorley, 2006).

RESULTS AND DISCUSSION

A distinct spatial and temporal heterogeneity in different water quality parameters was observed in the Vembanad estuary. During the study period, pH value in the estuary exhibited significant seasonal variation ($p<0.01$). The pH values recorded during the present study ranged between 6.96-7.32 on an average. Lowest pH of 6.25 was recorded in station 1 during the month of August and October in S1 and highest of 8.3 during February in station 10. These observed variations in pH could be ascribed due to relatively high land drainage and freshwater discharge from other perennial rivers (Satpathy *et al*., 2009). According to Neschuk *et al.* (2002) the best pH range for rotifers is 6.5-8.5. The rotifer occurrence in relation to pH and temperature was studied by Berzins and Pejler (1989). Average surface water temperature ranged from 29.4-30.8°C, with the highest value of 31.5°C during May and the lowest of 28°C during monsoon months. The ANOVA of temperature showed that variation between seasons was significant ($p<0.01$).

Mean dissolved oxygen concentration exhibited highest value of 8.13 mg L^{-1} in station 10 during July and lowest value of 4.18 in station 2 during December. All the sites showed average moderate dissolved oxygen content of 6.86±0.30 mg L^{-1}. The ANOVA of dissolved oxygen showed that variation between seasons was significant ($p<0.05$, $F = 16.67$). The dissolved oxygen of water expressed a negative correlation with the density and diversity of the majority of rotifer population. These results were in accordance with the studies of Saler and Sen (2002) and Sulehria and Malik (2012). Biological Oxygen Demand (BOD) values are moderately low in most of the stations ranging from 1.93-3.90 mg L^{-1} except in station 10, where it was about 4.9 mg L^{-1}. The BOD values showed station wise significance ($p<0.01$, $F = 3.3$). Low values of dissolved oxygen and elevated BOD values coincide with the low numerical density of rotifers at this site throughout the year.

The mean salinity values ranged between 0.48 ppt during monsoon to 25.86 ppt during post monsoon. The maximum salinity of 33 ppt was observed in station 10 and minimum of 0.01 ppt in station 2. The average salinity of 1.37±1.04 ppt was observed during the monsoon followed by pre monsoon (7.9±6.49 ppt) and post monsoon (7.89±6.12 ppt) periods. Salinity showed a significant positive correlation between pH ($r^2 = 0.327$, $p<0.01$) and temperature ($r^2 = 0.317$, $p<0.01$). The ANOVA showed that station wise and seasonal variations of salinity were significant. The annual average values of salinity showed that, the southern region of estuary was oligohaline (2.45 ppt) whereas, Northern region was mesohaline (11.85 ppt) in nature. Conductivity values in Vembanad estuary ranged between av.1.03-20.32 mS with lowest values in station 2 and highest in station 10. ANOVA of conductivity values exhibited significant variation at 1% level ($F = 4.27$). Conductivity expressed a significant negative correlation with rotifer density ($r = -0.268$) at 1% significance level during the present study.

Spatio-temporal variation of phosphate-phosphorus and nitrate-nitrogen values were significant, except for silicate-silicon. The nutrients in the Vembanad estuary originate from point sources like domestic and house boat sewage; industrial effluents etc., along with non-point sources like excess fertilizers agricultural field wash. Phosphate-phosphorous values recorded during the present study ranged between av. 1.28-2.88 μM L^{-1}. The ANOVA of phosphate-phosphorus values exhibited season wise significance ($p<0.01$, $F = 4.9$). Phosphate-phosphorus showed a positive correlation with rotifer density, which was in agreement with Kobayashi *et al.* (1998), who observed a positive correlation between total phosphorus and zooplankton density in Hawkesbury Nepean River in Australia where, 64% of total zooplankton taxa was composed of rotifers. Higher concentration of phosphate in the water may be attributed to enrichment by freshwater drainage (Mathew and Pillai, 1990).

Nitrate-nitrogen values varied between av. 1.30-1.87 μM L^{-1} with highest values in Southern stations (station 1, 2 and 3) in monsoon months owing to adjacent agricultural runoff. The ANOVA of nitrate-nitrogen showed season wise significance ($p<0.01$, $F = 2.203$). Nitrate-nitrogen values also exhibited a significant positive correlation with rotifer density at 1% level ($r = 0.269$). Mean seasonal values of silicate-silicon ranged between 15.5-24.8 μM L^{-1} with highest average values in stations 1 and 4 and lowest in station 8. Silicate-silicon did not exhibit any statistical significance. Chlorophyll a values ranged between 6.81-17.52 mg m^{-3}. The ANOVA of chlorophyll a did

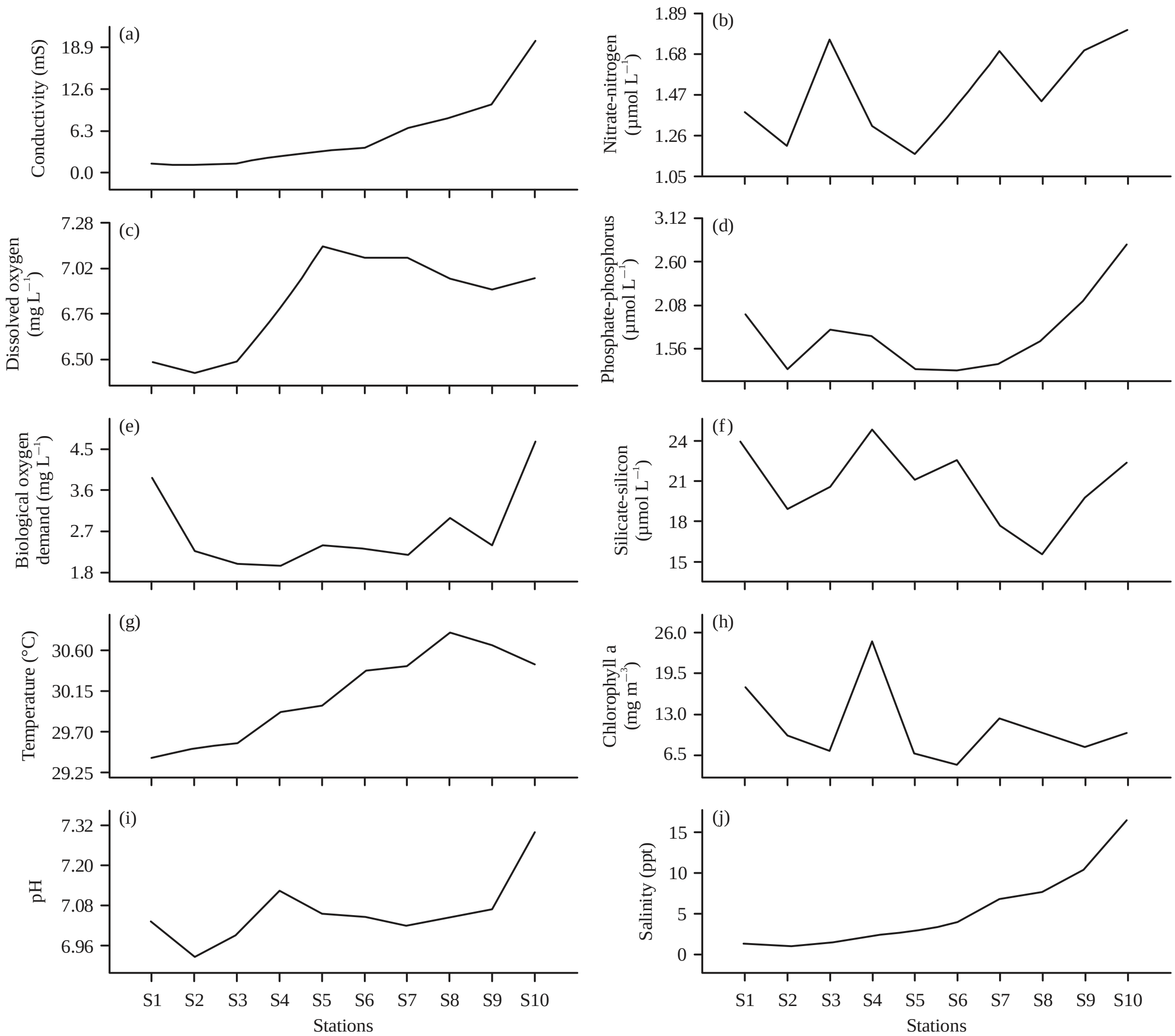

Fig. 2(a-j): Average variation in physico-chemical parameters, (a) Conductivity, (b) Nitrate-nitrogen, (c) Dissolved oxygen, (d) Phosphate-phosphorus, (e) Biological oxygen demand, (f) Silicate-silicon, (g) Temperature, (h) Chlorophyll a, (i) pH and (j) Salinity

not show any significant difference. Lack of significant correlation between chlorophyll a values and rotifer density may be due to their dependence on other alternative food sources such as detritus or organisms of the microbial pathway when phytoplankton is quantitatively or qualitatively insufficient (Gaudy *et al*., 1995). Station wise average variation in physico-chemical parameters are shown in Fig. 2.

Rotifers: The rotifer distribution showed a definite spatial and temporal variation in the estuary (Fig. 2) with highest abundance during monsoon months especially in Southern stations (station 1-4) of the Vembanad estuary. During March and April the average rotifer abundance was below 65 individual m^{-3}. Samples from different stations during different months indicated that distribution of rotifers was random. Fluctuation in their numbers may be due to keen competition between species for dominance. During the present investigation, 26 rotifer species were

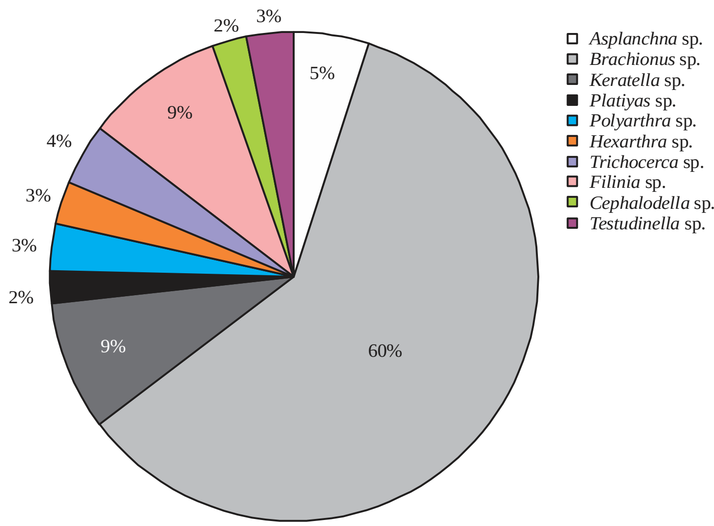

Fig. 3: Percentage composition of rotifers in Vembanad estuary during 2012-2013

encountered (Table 1) belonging to 10 genera under 2 orders. Planktonic rotifer communities in the freshwater parts of estuaries are often dominated numerically by members of the genera *Brachionus* and *Keratella* (Ferrari *et al*., 1989; Saunders and Lewis, 1989; Van Dijk and Van Zanten, 1995), whereas, species of the genus *Testudinella* are often the dominant form in the brackish-water regions. Among the various species encountered during the present study, the maximum diversity and abundance was seen in the *Brachionus* sp., represented by 19 different species which contributed 60% of the total rotifer density (Fig. 3) as previously studied by Gurav and Pejaver (2013) and Hamaidi-Chergui *et al*. (2013). Sharma (1983, 1987) also stated that, *Brachionus* sp., dominates in total rotifers in tropical regions and shows abundance in warmer parts of peninsular India which is in agreement with the present study. Brachionus is also an indicator of eutrophication (Bahura *et al*., 1993). Increase in rotifer density indicates advancing eutrophication (Gannon and Stemberger, 1978). Of the 26 species of rotifers, the predominant species were *Brachionus rotundiformis* (16.4%), *B. calyciflorus* (6.6%), *B. urceolaris* (5.5%), *B. plicatilis* (4.05%), *Keratella tropica* (3.2%) and *Asplanchna* sp. (2.2%) (Fig. 3).

Maximum abundance of *Brachionus rotundiformis* (4920 individual m^{-3}) was observed in station 8 during the month of January. Abundance of this particular species tends to decrease (0-40 individual m^{-3}) towards the month of June, July and August in study stations south of the Vembanad estuary. *Brachionus plicatilis* showed highest abundance of 1240 individual m^{-3} in station 1 during November. Among the 19 species of *Brachionus* recorded during the present study, *Brachionus rotundiformis* and *B. plicatilis* were observed from all the stations with varying salinity regimes which indicate the euryhaline nature of these two species. Salinity tolerance of these two species ranged between 8-12 and 5-15 ppt, respectively. *Brachionus plicatilis* was reported to be a euryhaline species by Sharma (1991). *Brachionus calyciflorus* species showed maximum abundance of 3640 individual m^{-3} in station 1 (Punnamada) during June and decreased towards northern stations during premonsoon and postmonsoon months. *Brachionus plicatilis* is considered as a common cosmopolitan species of alkaline waters and are numerically most significant. Agricultural runoff from nearby paddy fields and sewage discharge from houseboats, in Punnamada (station 1) provides a nutrient and organic rich eutrophic environment for

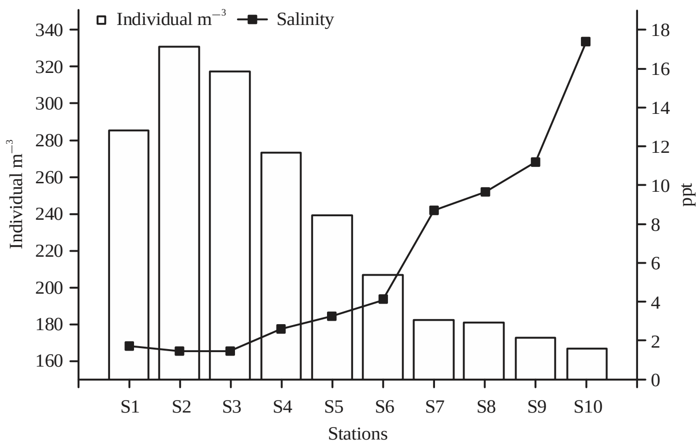

Fig. 4: Average spatial variation of rotifers and salinity in Vembanad estuary during 2012-2013

B. calyciflorus. According to Ferrari *et al*. (1989) this species is thought to be one of the rapidly thriving metazoans and a pioneer species, whose density increases with increase in waste water (Stevenson *et al*., 1998; Mukhopadhyay *et al*., 2007). *Brachionus quadridentata* and *B. calyciflorus* are considered to be representative of eutrophic conditions (Gannon and Stemberger, 1978; Sampaio *et al*., 2002). *Brachionus quadridentatus* increased to about 1600 individual m^{-3} in station 5 during July and was completely absent in stations 8, 9 and 10 throughout the study period, indicating its freshwater preference (salinity range 0.5-3 ppt). Maximum abundance of *B. urceolaris*, 1560 individual m^{-3} was recorded in the month of November in station 6 and declined to 0 in station 8, 9 and 10 during June and August. According to Sharma (1983), *B. angularis*, *B. calyciflorus*, *B. quadridentatus* and *B. caudatus* are widely distributed in India.

Asplanchna sp., abundance was found to highest (1040 individual m^{-3}) at station 2 and 5 during July and was present only in the upper reaches of the estuary which tends to decline during the summer months. *Filinia longiseta* was found to exhibit highest abundance (680 individual m^{-3}) in stations north of the estuary, mainly in stations 8 and 9 during April and May. According to Hutchinson (1967), *Filinia* is considered as summer dominant warm water species. It is also considered as an indicator of eutrophication (Maemets, 1983; Baloch *et al*., 2008). *Polyarthra vulgaris*, was found to occupy in stations enriched with organic pollution, with highest occurrence in station 3 (920 individual m^{-3}). The occurrence of species like *Filinia longiseta*, *B. forificula* and *Polyarthra vulgaris* are considered as indicator species which points out towards organic pollution and subsequent enriched status (Schindler and Noven, 1971; Rao and Durve, 1989). A substantial component of the diet of cyclopoid copepods includes *Polyarthra*, *Brachionus*, *Asplanchna*, *Synchaeta*, *Filinia* and *Keratella* spp. (Herzig, 1987). From this, it reflects that a good copepod population flourishes well, if only a rich population of rotifers exists and forms productive areas for rich fishery wealth (Varghese, 2006).

In general, rotifer abundance was higher in the freshwater dominated Southern stations, with a gradual declining trend towards the stations North of the estuary, indicating the prevailing salinity gradient (Fig. 4). The magnitude of salinity tolerance differed in different species of *Brachionus*. Nutrient enrichment due to sewage discharge and food availability due to heavy growth of phytoplankton could be the probable reasons for high rotifer density in the southern stations. The environmental parameters greatly influence the distribution and abundance of rotifer

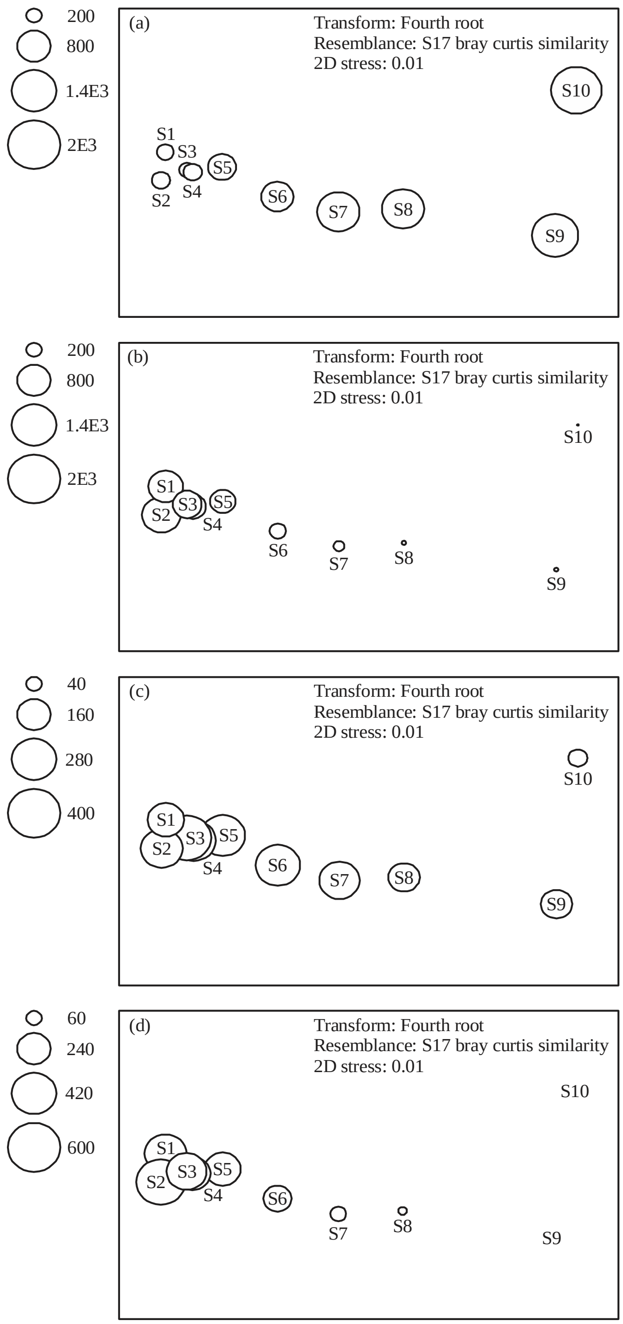

Fig. 5(a-d): MDS ordination plots of major rotifers, (a) *Brachionus rotundiformis*, (b) *Brachionus calyciflorus*, (c) *Brachionus plicatilis* and (d) *Brachionus forificula* in Vembanad estuary during 2012-2013

community of a particular aquatic ecosystem. Moreover, the trophic status and rotifer assemblages of an ecosystem are very much related (Kaushik and Saksena, 1995) and are largely governed by nutritional ecology of each species. Since, rotifers play an important role in the ecosystem, the ecological investigations on rotifers also gained importance.

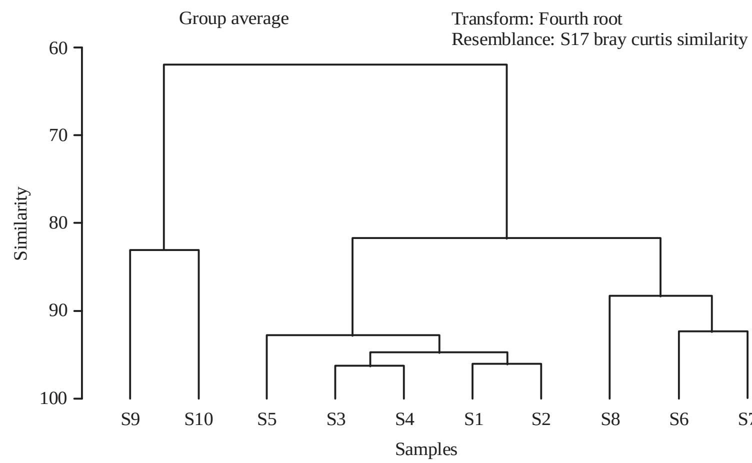

Fig. 6: Hierarchical cluster analysis of the rotifers in Vembanad estuary during 2012-2013

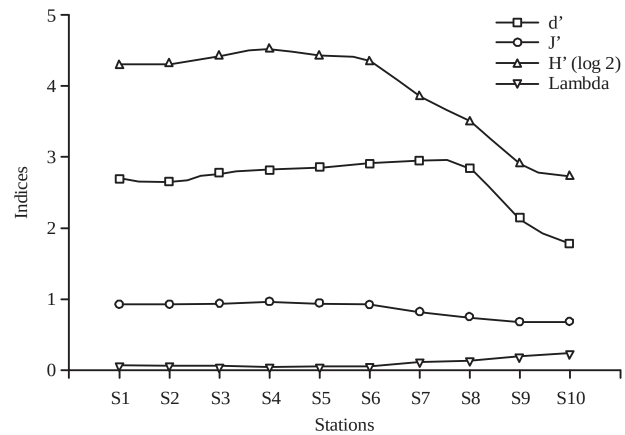

Fig. 7: Diversity indices of rotifers in Vembanad estuary during 2012-2013

Multi-Dimensional Scaling (MDS) ordination of rotifer distribution showed 80% similarity between stations 9 and 10 and between stations 1-7 with a stress value of 0.01. The MDS ordination of *B. rotundiformis*, *B. calyciflorus*, *B. plicatilis* and *B. forificula* is showed in Fig. 5. From the hierarchical cluster analysis of rotifer population in different stations it is evident that the stations south (station 1-5) of the Vembanad estuary are very similar implying its freshwater nature and formed separate cluster. Station 3 and 4 and 1 and 2 formed two separate clusters (cluster I and II) with 96.33 and 96.14% similarity. Cluster formed by station 8 along station 6 and 7 was at 88.3% similarity level. Station 9 and 10, which were on the Northern side of the estuary formed a separate cluster with 83.3% similarity due its brackish nature (Fig. 6).

Highest Shannon diversity index (4.51) was observed in southern stations, especially in station 3 (Rani) and lowest in station 10 (2.73). Salient feature of high species diversity in the Southern stations, with relatively low density of majority of the species may be attributed to fine niche partitioning amongst the species (Segers, 2008). The maximum richness index was observed in station 7 (2.95) and lowest in station 1 (1.72). Peilou's evenness was highest in station 4 (0.96) and lowest in station 9 and 10 (0.68). Rotifer diversity responds rapidly to changes in the aquatic environment. Higher diversity values coincide with higher evenness (Fig. 7).

Table 2: BEST results of environmental parameters and rotifer density

Variables	Variables selected	Best correlation values (Rho)
pH	5,6,9	0.860
Temperature	2,5,6,9	0.858
BOD	2,6,9	0.845
DO	2,5,9	0.845
Conductivity	2,5,6,8,9	0.838
Salinity	5,6,8,9	0.833
Chlorophyll a	5	0.829
Silicate	1,2,5,6,9	0.827
Phosphate	5,6	0.825
Nitrate	6,9	0.822

BOD: Biochemical oxygen demand, DO: Dissolve oxygen

Environmental matching (BEST), as per the PRIMER analysis (Table 2), showed that temperature, conductivity, salinity and phosphate were the best combination of variables that determined the abundance and distribution of rotifers in Vembanad estuary with a rho value of 0.86 (Table 2). Salinity gradient and conductivity are known to be important factors limiting the growth of planktonic rotifer populations in the Vembanad estuary, which was in agreement with the studies of Holst *et al*. (1998). Shiel (1979), while studying rotifers of the South Australia, also observed that no single factor can be defined as limiting, but increasing salinity had the most marked impact on the rotifer population, when a decrease in species diversity was recorded. Role of salinity in the distribution and occurrence of rotifers was also which was emphasized by Nair *et al*. (1984), Nair and Aziz (1987) and Gopakumar (1998) from Kerala. Kobayashi *et al.* (1998) also revealed a positive correlation with rotifer density with phosphate-phosphorus.

CONCLUSION

It is obvious from the results that, the population density and diversity of rotifers were strongly affected by the physicochemical parameters of the water body, either positively or negatively, throughout the period of study. It showed a significant negative correlation with temperature, pH and salinity ($p<0.01$) and significant positive correlation with nitrate-nitrogen ($p<0.01$), respectively. *Brachionus* sp., encountered the maximum diversity and density, comprising 19 different species contributing 60% of the total rotifer density. The predominant species were *Brachionus rotundiformis* (16.4%), *B. calyciflorus* (6.6%), *B. urceolaris* (5.5%), *B. plicatilis* (4.05%), *Keratella tropica* (3.2%) and *Asplanchna* sp. (2.2%). All dominant species observed in the main channel were eurytopic freshwater forms that became less abundant towards downstream (station 8, 9 and 10) which may be with respect to the salinity gradient prevailing in the estuary. Based on multivariate Bio-Env (BEST) analysis, temperature, conductivity, salinity and phosphate best determined the density and distribution of rotifers in the Vembanad estuary. The occurrence of like *Filinia longiseta*, *B. forificula* and *Polyarthra vulgaris* are considered as indicator species, which points out towards organic pollution and subsequent enriched status of the Southern stations of Vembanad estuary. As, the trophic status and rotifer assemblages of an ecosystem are very much related, a comprehensive study on the species diversity and community assemblage of rotifers in Vembanad estuary can furnish a valuable integrative index for further ecological assessment and monitoring of estuarine ecosystems.

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