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The Composition of Rotifers and Their Response to Environmental Factors Along the Hau River, Vietnam

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ABSTRACT

This study aimed to determine species composition, diversity, and distribution of rotifers in natural waters in order to select potential rotifer species that can be cultured for biomass to serve as live food for larviculture of economically important species toward diversifying aquaculture species in the Hau River, Vietnam. Sampling was carried out at 10 sites on the mainstream along the Hau River starting from the upstream (An Giang) to midstream (Can Tho, Hau Giang) and downstream (Soc Trang). The collection of the samples included water quality parameters and zooplankton including rotifers (Rotifera). The results showed that a total of 66 species of 31 genera were recorded in Hau River. The most commonly encountered species with high occurrence frequency in Hau River were *Polyarthra vulgaris*, *Brachionus rubens*, *B. calyciflorus*, *Filinia terminalis*, *Keratella cochlearis*, *K. valga* and *B. caudatus*. In Hau River, 57 species were found during the rainy season and 51 species in the dry season. A difference in species number was detected between sampling locations. The highest species number was found in the midstream areas, with 47 species recorded in the rainy season and 42 species in the dry season, followed by the upstream area with 39 species in the rainy season and 27 species in the dry season. The lowest species number was found in the downstream areas. The mean density of zooplankton was 15,895± 13,901 ind/m³ in which rotifers accounted for 55.45% (8,815±10,743 ind/m³). There was no significant difference in the density of zooplankton and rotifers between sampling areas during the rainy season. However, in the dry season, Thot Not had much higher zooplankton density than O Mon, Tra Noc, Ninh Kieu, and Cai Con, and significantly higher rotifer density than Chau Doc, Cai Con, and Tran De.

INTRODUCTION

The Mekong River Delta in Vietnam has the greatest potential for growth owing to advantageous circumstances such as good ecological conditions, natural resources, and effective policies. In 2019, the area constituted around 70% of the nation's aquaculture overall production **(Tri *et al.*, 2021)**. The Hau River (or Bassac River) is one of the two main Mekong River distributaries that flows into the Mekong Delta via Cambodia; the length is approximately 250 kilometers from the Cambodia border to the East sea **(Tran-Nguyen & Le, 2011; Renaud & Kuenzer, 2012)**. This is critical for the development of aquaculture in the Mekong Delta since it offers essential ecological services to local people **(Ut *et al.*, 2020)**.

Zooplankton is an essential component of the aquatic animal food chain, acting as an intermediary between phytoplankton and fish. Their functions are maintaining and improving a water body's biological production as well as assisting the ecosystem to remain balanced. In aquaculture, zooplankton is the main nutrient-dense food source that is essential for aquatic animals to consume when they are still at the larvae stage **(Lavens & Sorgeloos, 1996; Jeyaraj *et al.*, 2014)**. Rotifers are important groups of zooplankton. They are relatively small, short-lived, and fast-reproducing organisms **(C-P Stelzer, 2005)**. Many of them are known to play important roles and are widely distributed in aquatic ecosystems especially in fresh water, via the circulation of matter and energy transfer **(Wetzel, 2001; Balian *et al.*, 2008; Yin *et al.*, 2018; Jurczak *et al.*, 2019)**. They contribute to the biodiversity of tropical and equatorial water bodies and streams **(Dussart *et al.*, 1984)**. Furthermore, rotifer species composition is vulnerable to environmental changes, its ecological features can be used as biological monitoring to evaluate the quality of the aquatic environment, and it reflects the nutritional status of the ecosystem **(Crivelli *et al.*, 1997; Dahms *et al.*, 2011; Pan *et al.*, 2016)**. Several studies highlighted changes in various environmental factors, which reflect alterations in population dynamics, community structure, and the functions of rotifers **(Yin *et al.*, 2018; Yu *et al.*, 2019; Liang *et al.*, 2020)**.

This study was conducted to investigate the species composition and distribution of rotifers, as well as their relationships with environmental factors along the Hau River. The findings aimed to serve as a database for further research on rotifers and to enhance the understanding and identification of potential species for aquaculture in this region. To the best of our knowledge, this is the first report on the community structure of rotifers along the Hau River.

MATERIALS AND METHODS

1. Sampling sites and time

Zooplankton samples were collected from the water bodies along the Hau River, covering the upstream (from Chau Doc to the Thot Not border), midstream (from Thot Not to the end of Hau Giang Province), and downstream (Soc Trang area) sections (Table 1 & Fig. 1). Each segment is approximately 67km long. Samples were gathered at the collection site following a river's cross-section, with three distinct points: two along the opposite banks and one in the river's middle. The Supplemental Table (1) shows the longitude and latitude coordinates for each sampling location.

Sampling in this study was carried out every three months from March to December, with a total of four occasions as follows: the first in the middle of the dry season (March); the second at the end of the dry season and the beginning of the rainy season (June); the third in the middle of the rainy season (September); and the fourth at the end of the rainy season and the beginning of the dry season (December).

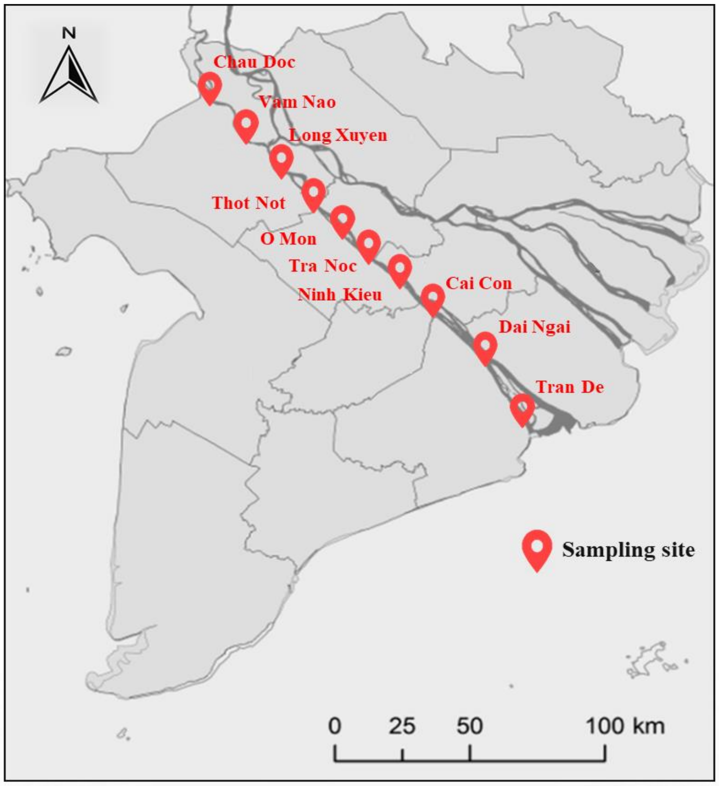

Fig. 1. Hau River sampling sited (Mekong delta, Vietnam)

Table 1. Distribution of sampling locations in Hau River

Sampling areas	Sub-areas *	Locations	Number of sampling locations
Natural water bodies In Hau river	Upstream	Chau Doc, Vam Nao, Long Xuyen	9
	Midstream	Thot Not, O Mon, Tra Noc, Ninh Kieu	12
	Downstream	Cai Con, Dai Ngai, Tran De	9
Total			30

(*) Upstream: from Chau Doc to Thot Not border,
Midstream: from Thot Not to the end of Hau Giang Province,
Downstream: in Soc Trang area,
Each segment is about 67km long.

Supplemental Table 1. The coordinate of sampling sites along the Hau River

No	Segment	Sampling site	Code	Coordinates	
				Latitude (E)	Longitude (N)
1	Upstream	*Chau Doc* 1	CD1	10.7112 °N	105.1257 °E
2		*Chau Doc* 2	CD2	10.7103 °N	105.1248 °E
3		*Chau Doc* 3	CD3	10.7098 °N	105.1227 °E
4		*Vam Nao* 1	VN1	10.5561 °N	105.2879 °E
5		*Vam Nao* 2	VN2	10.5541 °N	105.2874 °E
6		*Vam Nao* 3	VN3	10.5526 °N	105.2868 °E
7		*Long Xuyen* 1	LX1	10.3950 °N	105.4388 °E
8		*Long Xuyen* 2	LX2	10.3935 °N	105.4376 °E
9		*Long Xuyen* 3	LX3	10.3917 °N	105.4371 °E
10	Midstream	*Thot Not* 1	TN1	10.2686 °N	105.5401 °E
11		*Thot Not* 2	TN2	10.2692 °N	105.5405 °E
12		*Thot Not* 3	TN3	10.2700 °N	105.5413 °E
13		*O Mon* 1	OM1	10.1538 °N	105.6620 °E
14		*O Mon* 2	OM2	10.1511 °N	105.6579 °E
15		*O Mon* 3	OM3	10.1463 °N	105.6562 °E
16		*Tra Noc* 1	TN1	10.1113 °N	105.7280 °E
17		*Tra Noc* 2	TN2	10.1051 °N	105.7252 °E
18		*Tra Noc* 3	TN3	10.0976 °N	105.7223 °E
19		*Ninh Kieu* 1	NK1	10.0395 °N	105.8001 °E
20		*Ninh Kieu* 2	NK2	10.0406 °N	105.7980 °E
21		*Ninh Kieu* 3	NK3	10.0406 °N	105.7948 °E
22	Downstream	*Cai Con* 1	CC1	9.9413 °N	105.9018 °E
23		*Cai Con* 2	CC2	9.9397 °N	105.8994 °E
24		*Cai Con* 3	CC3	9.9324 °N	105.8947 °E
25		*Dai Ngai* 1	DN1	9.7375 °N	106.0761 °E
26		*Dai Ngai* 2	DN2	9.7357 °N	106.0754 °E
27		*Dai Ngai* 3	DN3	9.7335 °N	106.0752 °E
28		*Tran De* 1	TD1	9.5322 °N	106.2152 °E
29		*Tran De* 2	TD2	9.5289 °N	106.2096 °E
30		*Tran De* 3	TD3	9.5265 °N	106.2020 °E

2. Methodology

2.1. Water quality samples collection

Water quality factors including 15 parameters were measured simultaneously with collecting zooplankton samples from the water bodies. The water quality parameters were assessed (directly measured, preserved samples brought to the laboratory) and analyzed (specialized instruments and standard methods) according to the methods shown in Supplemental Table (2).

Supplemental Table 2. Sampling and analytical methods for environmental parameters

No.	Parameters	Sampling methods	Analytical methods
1	Temperature (°C)	Direct measurement	HANNA HI98194 Multiparameter
2	pH	Direct measurement	HANNA HI98194 Multiparameter
3	Clarity (cm)	Direct measurement	Secchi Disk
4	Salinity (PPT)	Direct measurement	Refractometer
5	Water velocity (m/s)	Direct measurement	Flowmeter
6	Dissolved oxygen (DO, mg/L)	Direct measurement	HANNA HI98194 Multiparameter
7	BOD_5 (mg/L)	Collect the water sample in a dark 125 ml glass bottle and incubate at 20°C.	5210-5 day BOD test
8	COD (mg/L)	Collect the water sample in a 125 ml glass bottle, then fix the sample with H_2SO_4 4M	Closed reflux 5220 B
9	TAN (mg/L)	Collect water sample in a 1l plastic bottle and refrigerate (4°C).	Phenate
10	$N\text{-}NO_2^-$ (mg/L)	Collect water sample in a 1l plastic bottle and refrigerate (4°C).	Diazonium Method 4500-NO_2^-
11	$N\text{-}NO_3^-$ (mg/L)	Collect water sample in a 1l plastic bottle and refrigerate (4°C).	Colorimetric method, Salicylate 4500-NO_3^-
12	$P\text{-}PO_4^{3-}$ (mg/L)	Collect water sample in a 1l plastic bottle and refrigerate (4°C).	Colorimetric method, $SnCl_2$ 4500-P-D method

13	Total Dissolved Solids (TDS, mg/L)	Collect water sample in a 1l plastic bottle and refrigerate (4°C).	Filter through 0.45 µm glass microfiber filters and dry at 180°C (2540 C. TDS).
14	Total Suspended Solids (TSS, mg/L)	Collect water sample in a 1l plastic bottle and refrigerate (4°C).	Filter through 0.45 µm glass microfiber filters and dry at 103°C (2540-D. TSS).
15	Chlorophyll-a	Collect water sample in a 1l plastic bottle and refrigerate (4°C).	Extraction with acetone and colorimetric method.

2.2 Zooplankton samples collection

The qualitative and quantitative methods for collecting rotifer samples at a depth of 30 centimeters both involved using a plankton net with a mesh size of 60µm. The qualitative method for zooplankton collection was described in a previous study **(Nguyen *et al.*, 2020)**. At the collection site, samples were gathered by dragging the net for approximately 5 minutes, with an average pulling speed of about 0.5 m/s. The collected samples were stored in 110ml plastic bottles and preserved with 4% formalin. Species composition was determined based on morphological and taxonomic characteristics, **(Shirota, 1966; Dang *et al.*, 1980; Harris, 2005)**.

Quantitative samples of zooplankton were collected at each sampling point by taking 200 liters of water by Niskin water sampler (10 liters) and filtering the collected water through a plankton net. Samples were stored in 110ml plastic bottles and fixed with formalin as described above. The volume of water after filtration through the mesh was measured using a graduated cylinder. Then, 1ml of the concentrated sample was placed in the Sedgwick-Rafter counting chamber, and species were counted by moving from top to bottom and left to right under the microscope. Counting was done three times, with 60 random cells counted each time. The zooplankton density was determined using the formula of **Lenore *et al*. (1999)**.

$$\mathbf{X\ (individual/m^3)} = \frac{\mathbf{C \times 1000 \times V_c \times 10^6}}{\mathbf{V_1 \times N \times V_t}}$$

Where,

C: number of individuals counted in the counting chamber

N: total count cells (180 cells)

V_1: volume of a counting cell (1 mL)

Vc: determined volume water sample before counting (mL)

Vt: volume of water sample (200 mL)

3. Biodiversity index

The abundance and diversity of rotifers in water bodies and at sampling sites were assessed through the following parameters:

Shannon-Wiener diversity index: This index measures species richness and the uniformity of individual distribution, reflecting the complexity and stability of community structure: $\mathbf{H' = -\Sigma pi.lnpi}$

Simpson's diversity index: This index accounts for both the number of species and their relative abundance: $\mathbf{D = 1 - \sum N_i (N_i-1)/N(N-1)}$

Margalef Richness Index: This index measures species richness and the number of individuals: $\mathbf{J' = (S-1)/lnN}$

Lambda dominant species index: This index quantifies the dominance of species in the community: $\boldsymbol{\lambda = \sum P_i^2}$

Where, $P_i = n_i/N$; n_i is the density of the i^{th} species in one site; N is the total density; S is the total number of species.

4. Data analysis

The data were analyzed by SPSS 25 statistical software; T-test was used to evaluate and compare the difference of indicators; Pearson correlation comparison was used to evaluate the correlation between environmental factors with the density of zooplankton and the density of rotifers in the Hau River.

Correlation between environmental factors and the density of zooplankton and the density of rotifers in the Hau River, accumulation and species rank of zooplankton and rotifera along the Hau River were compared and evaluated based on multivariate statistical analysis using PRIMER V.6.1 software (Plymouth Routines in Multivariate Ecological Research) **(KR Clarke & RN Gorley, 2006)**. The distribution of rotifera was evaluated through cluster analysis according to **Legendre (1998)** and CCA (Canonical correlation analysis) correlation according to **Legendre and Gallagher (2001)** using R.3.6.0 software combined with R.studio 1.2.1335.

RESULTS

1. Water quality

Water quality evaluation along the Hau River is listed in Table (2). The temperature at the sampling sites during the rainy and dry seasons in the sampling areas ranges from 28.7± 0.4 to 30.2± 0.3°C on average.

The pH value does not differ much between seasons of the year in the sampling areas; the highest was recorded for the downstream area in the rainy season (7.3±0.5) and the lowest for the upstream area in the rainy season (6,4±0.1). Clarity in the rainy season is higher than in the dry season. In the dry season, salinity in the downstream area, especially at Dai Ngai and Tran De sampling areas, increases to 5 and 2ppt and decreases to 3 and 0ppt in the rainy season, respectively. In the rainy season, the mean flow rate is 1.7± 3.0m/ min, much higher than the dry season of 0.6± 0.8m/ min, especially in the upstream. The mean PO_4^{3-} concentration in the rainy season is also higher than in the dry season, with values of 0.24± 0.15 and 0.10± 0.09mg/ L, respectively. In contrast, the average concentrations of TAN and NO_3^- in the rainy season are lower than those in the dry season, with 0.633± 0.849, 0.274± 0.089mg/ l and 0.893± 0.848, 0.352± 0.153mg/ l, respectively. The TSS concentration in the rainy season (132.4± 97.5mg/ l) is higher than in the dry season (47.4± 40.95mg/ l).

In the Hau River, the mean dissolved oxygen (DO) concentration is 5.0 ± 1.1mg O_2/l during the rainy season and 5.7 ± 0.5mg O_2/l in the dry season, with no significant differences between sampling areas (ranging from 4.9 ± 0.5 to 5.5 ± 0.4mg O_2/l). The average chlorophyll-a content is higher in the dry season (18.4 ± 7.2µg/ l), approximately twice that of the rainy season (9.1 ± 3.4µg/ l). The results indicate that chlorophyll-a concentrations are lower at the midstream collection site compared to the other two regions. Additionally, elevated phosphate concentrations were observed at ten specific sites, which may serve as an indirect nutrient source, promoting an increased zooplankton growth.

Table 2. Environmental data (mean±SD) for the different regions and periods in Hau River, in rainy and dry season

Parameter	**Rain season**			**Dry season**		
	Upstream	Midstream	Downstream	Upstream	Midstream	Downstream
Temperature (˚C)	30,2±0,3	29,9±0,2	29,9±0,5	28,0±0,4	29,0±1,1	28,7±0,3
pH	6,4±0,1	6,9±0,7	7,3±0,5	6,7±0,1	6,8±0,4	6,4±0,2
Clarity (cm)	40,6±5,5	37,5±8,1	30,5±11	29,4±5,1	34,7±3,9	21,2±7,3
Salinity (PPT)	-	-	0-3	-	-	0-5
Flow rate (meter/min)	4,9±4,0	1,6±2,7	3,8±3,9	8,3±5,9	4,4±7,0	3,4±3,5
DO (mgO_2/L)	4,9±0,5	5,6±1,1	5,5±0,8	5,8±0,4	5,9±0,4	5,2±0,7
BOD_5 (mgO_2/L)	3,7±0,4	4,2±0,8	4,1±0,6	4,3±0,3	4,4±0,3	3,9±0,5
COD (mgO_2/L)	5,6±1,1	5,41,8	12,5±4,8	8,9±1,7	6,8±1,9	11,3±4,5
TAN (mg/L)	0,68±0,42	0,54±0,59	0,71±0,3	0,96±0,5	0,75±0,37	0,86±0,23
NO_2^- (mg/L)	0,05±0,03	0,04±0,03	0,07±0,04	005±0,02	0,05±0,03	0,01±0,01
NO_3^- (mg/L)	0,25±0,1	0,27±0,2	0,31±0,1	0,1±0,03	0,2±0,01	0,34±0,08
PO_4^{3-} (mg/L)	0,25±0,06	0,21±0,06	0,31±0,1	0,18±0,1	0,22±0,16	0,05±0,04
TDS (mg/L)	291±48	227±56	739±740	179±47	142±44	420±344
TSS (mg/L)	34,3±9,4	55,3±23,1	80±29,5	130±41	105±51,5	95,4±34,5
Chlorophyll-*a* (µg/L)	12,9±5,2	10,1±3,3	5,1±0,9	19,1±9,0	14,8±2,9	16,9±1

2. Composition species of rotifer in Hau River

The composition species of rotifera in the Hau River was recorded with 66 species belonging to 31 genera and 12 families of 2 classes, *Bdelloidea* and *Monogononta* (Supplemental Table 3). Some rotifers species were found up, mid and downstream of the Hau River, including *Brachionus rubens, B. calyciflorus, B. falcatus, B. caudatus, B. plicatilis, Filinia terminali, Keratella cochlearis, K. valga, Polyarthra* sp., and *P. vulgaris*. This result is consistent with that of **Bekelegen (2001)** who postulated that *Keratella* and *Brachionus* are the dominant groups in the river under study.

The findings indicate that in the upstream region, there are 46 species of rotifers belonging to 22 different genera (Table 3). Some species are present in this area, including *P. vulgaris, B. rubens, B. falcatus, B. calyciflorus, F. terminalis, K. cochlearis,* and *K. valga*. 10 species belong to *Brachionus* genera, especially *Brachionus ruben* and *B. falcatus* which are present at almost every sampling region. This result is similar to the the finding of **Sladecek (1983)** since those species are typically found in freshwater environments. There were 7 species of *Keratella* genera found in this study, which are all present in this area.

In the midstream, the *Brachionus* genus has the most species (13 species), with *Brachionus rubens, B. calyciflorus,* and *B. falcatus* being frequently found in this area. Additionally, this area also has 7 species from *Keratella* genera (Table 3).

The downstream area includes the lowest rotifer species compared to the middle and the upstream areas, which include 41 species belonging to 17 genera. Some of the most common species found here were *Brachionus plicatilis, B. calyciflorus, B. rubens, B. caudatus, B. calyciflorus,* and *Polyarthra vulgaris.*

The results of cluster analysis considering the similarity of the rotifer composition in the sampling areas along the Hau River, with 10 sampling collection sites, showed that 9 clusters are similar in the distribution of rotifer species in the distance of 1-25 (Euclidean distance: 10,517-15,881) (Supplemental Table 4). The similarity in rotifer species composition has the highest value at the collection points of Cai Con and Dai Ngai sampling sites (cluster 1 [8-9]) (Fig. 2), with a distance of 1 and a Euclidian value of 10,571-10,898. The next sampling site with a similar value is the Tran De [10] compared with cluster 1 [8-9] (Fig. 2). When the distance is the furthest, it shows the lowest similarity for 2 sampling sites, Tra Noc [6] and Thot Not [4], with values from 19-25 and large Euclidian values from 14.471-15,881, compared to other sites. This shows that for the sampling sites in brackish water, the composition of rotifera has a high degree of similarity and diversity compared to the sampling site in freshwater. The sampling sites in freshwater areas have average similarity values and more diversity in rotifer composition. The results show that the sampling site has a great impact on the species composition and rotifer diversity along the Hau river route during the time of the sampling.

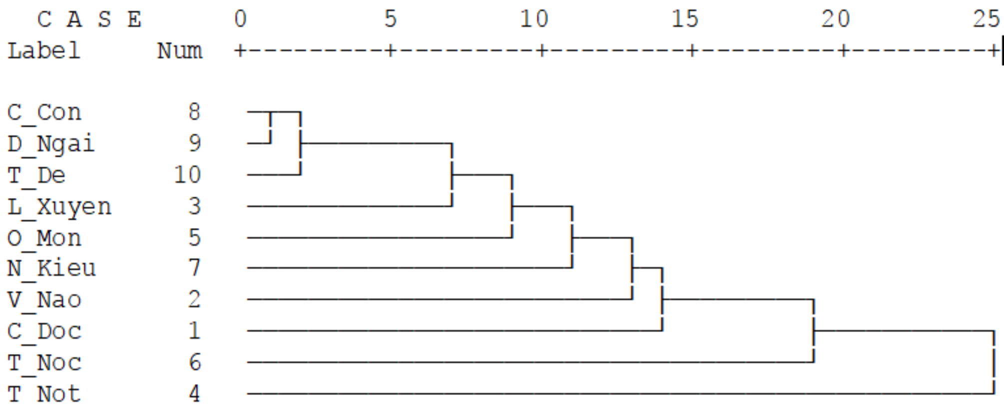

Fig. 2. Cluster analysis of the rotifer composition along the Hau River

Table 3. Rotifers species recorded along the Hau River

No	Species	Frequency of occurrence											
		Upstream				Midstream				Downstream			
		1st time	2nd time	3rd time	4th time	1st time	2nd time	3rd time	4th time	1st time	2nd time	3rd time	4th time
1	*Albertia typhylina*				*				*			*	
2	*Anuraeopsis fissa*							*					
3	*Ascomorpha ecaudis*		*			*	*	*		*			
4	*Ascomorphella volvocicola*									*			*
5	*Asplanchna priodonta*	*				*	*			*	*		
6	*Asplanchnopus myrmeleo*		*			*				*			
7	*Brachionus angularis*	*	*			*	*	*	*				*
8	*Brachionus bakeri*		*			*							
9	*Brachionus bidentata*						*						*
10	*B.budapestinensis*												*
11	*Brachionus diversicornis*					*	*					*	
12	*Brachionus calyciflorus*	*	*		*	*	*	*	*	*	*	*	*
13	*Brachionus caudatus*		*	*	*	*	*	*	*	*	*	*	*
14	*Brachionus falcatus*	*	*	*	*	*	*	*	*	*		*	*
15	*Brachionus forficula*		*	*			*					*	
16	*Brachionus genus*					*		*					
17	*Brachionus havanaensis*									*			
18	*Brachionus pala*						*						
19	*Brachionus patulus*							*	*				
20	*Brachionus plicatilis*	*	*			*	*	*		*	*	*	*
21	*Brachionus quadridentata*				*			*	*	*			*
22	*Brachionus rubens*	*	*	*	*	*	*	*	*	*	*		*
23	*Cephalodella auriculata*	*											
24	*Colurella adriatica*					*							
25	*Conochilus unicornis*							*					
26	*Dicranophorus forcipatus*								*				
27	*Dipleuchlanis propatula*			*		*	*						
28	*Elosa woralli*		*				*						
29	*Encentrum felis*						*	*	*				

No	Species	Frequency of occurrence											
		Upstream				Midstream				Downstream			
		1st time	2nd time	3rd time	4th time	1st time	2nd time	3rd time	4th time	1st time	2nd time	3rd time	4th time
30	*Embata commensalis*		*										
31	*Epiphanes brachionus*		*				*						
32	*Epiphanes senta*	*	*			*	*						
33	*Euchlanis dilatata*	*				*	*		*				
34	*Filinia brachiate*		*	*			*	*	*			*	
35	*Filinia opoliensis*							*					*
36	*Filinia terminalis*	*	*	*	*	*	*	*	*		*	*	*
37	*Hexarthra mira*									*			
38	*Keratella cochlearis*	*	*	*	*	*	*	*	*			*	*
39	*Keratella hiemalis*	*	*	*		*	*			*		*	
40	*Keratella quadrata*			*				*					*
41	*Keratella serrulata*	*		*	*		*	*	*	*		*	*
42	*Keratella stipitata*	*	*	*		*		*			*	*	
43	*Keratella tropica*	*				*							
44	*Keratella valga*	*	*	*	*	*	*	*	*		*	*	*
45	*Lecane nitada*				*		*		*			*	
46	*Lecane luna*		*			*	*	*		*	*	*	
47	*Lecane elasma*					*	*						
48	*Lecane ohioensis*											*	
49	*Lepadella ovalis*						*					*	
50	*Lepadella patella*					*							
51	*M.quadricornifera*	*	*			*	*				*		
52	*Monostyla bulla*	*	*	*		*	*			*	*		
53	*Monostyla lunaris*		*	*		*	*	*	*		*		
54	*Monostyla quadridentata*		*								*		
55	*Philodina roseola*	*	*			*	*			*	*		
56	*Phompholyx sulcata*	*				*	*						
57	*Platyias patulus*	*	*		*	*	*		*	*	*		
58	*Platyias quadricornis*		*				*						
59	*Ploesoma lenticulare*							*					
60	*Ploesoma triacanthum*			*									
61	*Ploesoma truncatum*			*								*	
62	*Polyarthra euryptera*										*		
63	*Polyarthra* sp.	*	*	*	*	*	*	*	*	*		*	*
64	*Polyarthra vulgaris*	*	*	*	*	*	*	*	*	*	*	*	*
65	*Trichocerca longiaeta*			*				*	*				
66	*Trichocerca cylindrical*			*	*			*				*	

Presence (*) and absence ( ) of rotifer species.

Supplemental Table 4

Species composition of each sampling site	Distance (Euclidian)	Distance [1-25]	Sampling sites
1	10,517	1	1,2,3,4,5,6,7,(8,9),10
2	10,898	2	1,2,3,4,5,6,7,(8,9,10)
3	11,871	7	1,2,4,5,6,7,(8,9),(3,10)
4	12,245	9	1,2,4,6,7,(8,9),(3,5,10)
5	12,759	11	1,2,4,(5,7),6,(8,9),(3,10)
6	13,131	13	1,(2,7),4,6,(8,9),(3,5,10)
7	13,495	14,5	(1,2),4,6,(8,9),(3,5,7,10)
8	14,471	19	(1,2,6),4,(8,9),(3,5,7,10)
9	15,881	25	(1,2,3,4,5,6,7,8,9,10)

Where: 1-Chau Doc, 2-Vam Nao, 3-Long Xuyen, 4-Thot Not, 5-O Mon, 6-Tra Noc, 7-Ninh Kieu, 8-Cai Con, 9-Dai Ngai, 10-Tran De.

3.1. Density of the rotifera in the Hau River

The zooplankton density along the Hau River was not significantly different in the rainy season; however, a high fluctuation was recorded between sampling sites throughout the dry season (Fig. 3). In the rainy season, the highest zooplankton density was Vam Nao and the lowest density was Dai Ngai, with the former density being twice as much as that of the latter (20064.7 ind/m^3 and 9081.95 ind/m^3, respectively). In the dry season, at Thot Not the zooplankton density was the highest compared to other sampling sites, as well as significantly higher compared to O Mon, Tra Noc, Ninh Kieu and Cai Con. Thot Not had a density of nearly quintuple compared to the lowest (31116.45 ind/m^3 and 6516.56 ind/m^3, respectively).

As shown in Fig. (3), at eight out of ten sampling sites along the Hau River, the zooplankton density in the rainy season is usually higher than in the dry season, and rotifer resembles the majority of zooplankton density compared to other groups. The exception to this was shown in the dry season at the Dai Ngai and a greater difference at Tran De, where the density of copepod was higher than rotifer. On the other hand, Thot Not rotifer density in the dry season accounts for 89.5% of the overall density, and it also has the highest rotifer density in the Hau River with a density of 27536.86 ± 27395.79 ind/m^3 (Fig. 4). Additionally, it was significantly higher in density than at Chau Doc, Cai Con and Tran De (4067.95±1484.49 ind/m^3, 3984.08±4134.45 ind/m^3, 4745.81±9011.68 ind/m^3, respectively).

In Chau Doc province, the density of rotifers in the rainy season is 11,730±11,062 inds/m^3, which is nearly 3 times higher than that in the dry season of 4,067±1,484 inds/m^3. In Vam Nao province, the density of rotifers in the rainy season is also higher than in the dry season at 13,168±12,556 inds/m^3 and 9,962±12,634 inds/m^3, respectively (Fig. 4).

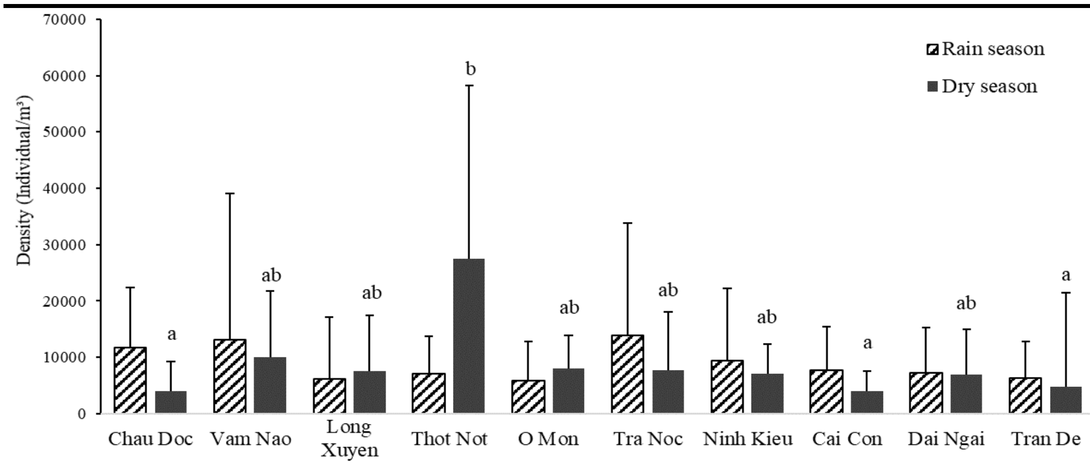

Fig. 3. The average density of the zooplankton along Hau River in dry and rain season The data are expressed as mean ± SD (n = 6). Significant differences of the density in the dry season (*P*< 0.05, one-way ANOVA and the Tukey's post hoc test) are indicated by different letters

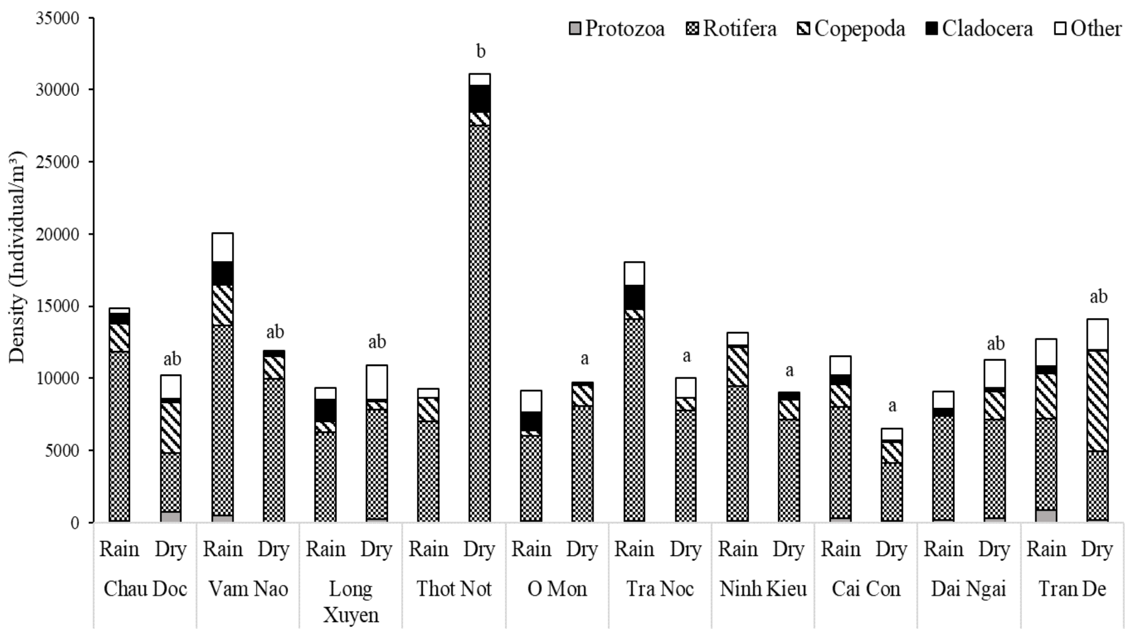

Fig. 4. Density of the zooplankton along Hau River in dry and rain season. The data are expressed as mean ± SD (n = 6). Significant differences of the density in the dry season (*P*< 0.05, one-way ANOVA and the Tukey's post hoc test) are indicated by different letters

3.2. Density of the rotifers along Hau River distributed in clusters

Analyzing clusters of rotifer density at 10 sampling sites along the Hau River shows that there are 9 clusters with distances from 1-25, and the Euclidian distance value is from 8,245-14,695 (Supplemental Table 5). The results show that the similarity in rotifer density with the highest value is at the two sampling sites, O Mon and Tra Noc

(Fig. 5), with a similarity distance of 1 and obtained an Euclidian value of 8,245-8,789. The remaining sampling sites have lower similarities in terms of rotifer density and the distance value has an average distance of 3, the Euclidian value has an average distance of 806 across the homologous clusters. The analysis results show that the Tran De sampling site [10] has the lowest density of rotifers compared to other sites, with a distance value of up to 25 and an Euclidian value of 14,695.

The cluster analysis results also show that in addition to the Tran De sampling site [10], the sampling sites in cluster 8 [1,2,4,9], Chau Doc, Vam Nao, Thot Not and Dai Ngai, also have similar low rotifer density values (Fig. 5). The homologous density of rotifers is the highest in cluster 2 [5,6,8] through 3 sampling site, namely O Mon, Tra Noc and Cai Con province, during time of sampling. This shows that when using herarchical cluster analysis software to analyze clusters about the similarity of rotifer density at sampling sites along the Hau River, the location and sampling sites have a great impact on the density and abundance of rotifers in the river.

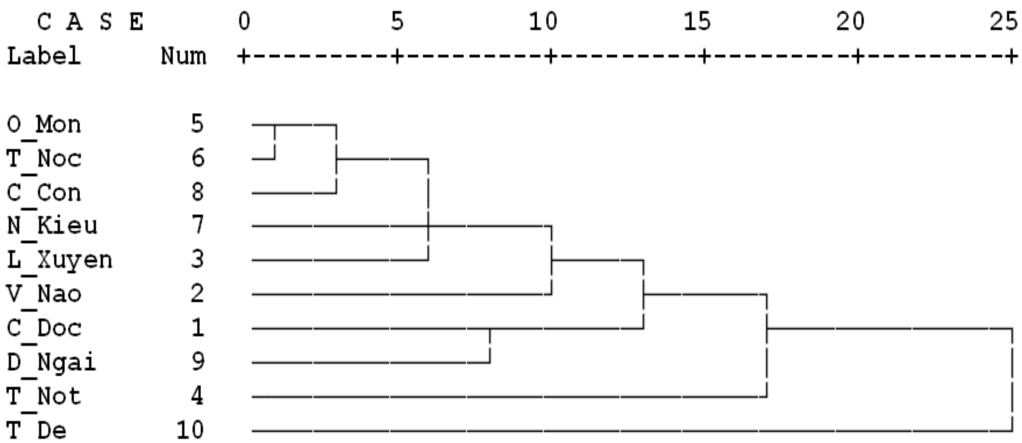

Fig. 5. Cluster analysis of the rotifer density distributed along Hau River

Supplemental Table 5

Species composition of each sampling site	Distance (Euclidian)	Distance [1-25]	Sampling sites
1	8,245	1	1,2,3,4,(5,6),7,8,9,10
2	8,789	3	1,2,3,4,(5,6,8),7,9,10
3	9,650	6	1,(2,3,7),4,(5,6,8),9,10
4	9,777	7,5	(1,9),2,(3,7),4,(5,6,8),10
5	10,127	10	(1,9),(2,3,7),4,(5,6,8),10
6	10,698	13	(1,9),(2,3,7),4,(5,6,8),10
7	11,569	17	(1,2,4,9),(3,5,6,7,8),10
8	12,481	20	(1,2,4,9),(3,5,6,7,8),10
9	14,695	25	(1,2,3,4,5,6,7,8,9,10)

Where: 1-Chau Doc, 2-Vam Nao, 3-Long Xuyen, 4-Thot Not, 5-O Mon, 6-Tra Noc, 7-Ninh Kieu, 8-Cai Con, 9-Dai Ngai, 10-Tran De.

4. Biodiversity indicators

Fig. (6) shows the relationship between cumulative dominance (%) and species rank of zooplankton groups in water bodies in the Hau River. The results of the analysis have shown a relatively clear difference in the accumulation and species ranking between rotifera and other zooplankton groups (Protozoa, Cladocera, Copepoda, and others) in the Hau River. At the Hau River sampling sites, rotifera is the group with the slowest increase in species rank and protozoa is the group with the highest cumulative increase in index.

The analysis results of the biodiversity indicators of the zooplankton community in the Hau River (Table 4) spotted a difference in the richness of Magalef species (D) and the Shannon diversity index (H') of 4 groups of zooplankton in different types of water bodies. It is easy to see that the richness and diversity of the rotifera are quite high in the type of flowing water bodies (river) with the highest index of 8.36 (D) and 4.23 (H'), respectively. The Pielou similarity index (J') presented in Table (4) clarified that there is no big difference between the wildlife groups in the Hau River (J' ranges from 0.89 to 0.95).

In other words, the community of zooplankton species in the Hau River is highly uniform and relatively stable. The 1-Lambda index (λ) presented in Table (4) shows that the dominant species diversity is higher in the water bodies of the Hau River (0.932-0.979). Thus, it can be concluded that with the characteristics of water sources, water quality and the impact of flows have created an abundance of zooplankton in the Hau river.

Regarding the difference in the number of species, the analysis results show that in the water bodies of the river, the rotifera dominates both in terms of species richness and diversity ((d) and (H') reach the highest value, 8.36 and 4.23, respectively). Two groups of zooplankton that always have the lowest species richness and diversity in all water bodies are Protozoa and Cladocera. Regarding the diversity of dominant species, rotifera is always the leading group with the highest number of 1-Lambda (λ) (λ = 0.979).

More specifically, between 10 locations in Hau River, Chau Doc has the highest biological indicator including richness index (D), diversity index (H'), Similarity (J'), and Dominance (L) (Fig. 7), with D=2.97, J'= 0.94, H'=3.30, λ=0.954, respectively. The similarity index was not much different between those locations with values ranging from 0.83 to 0.94 (except for the rotifer population in Thot Not area with J'=0.63). Although the richness of rotifer in Thot Not is quite high (D=2.76), the species diversity, similarity, and dominance had the lowest value compared to the rest locations.

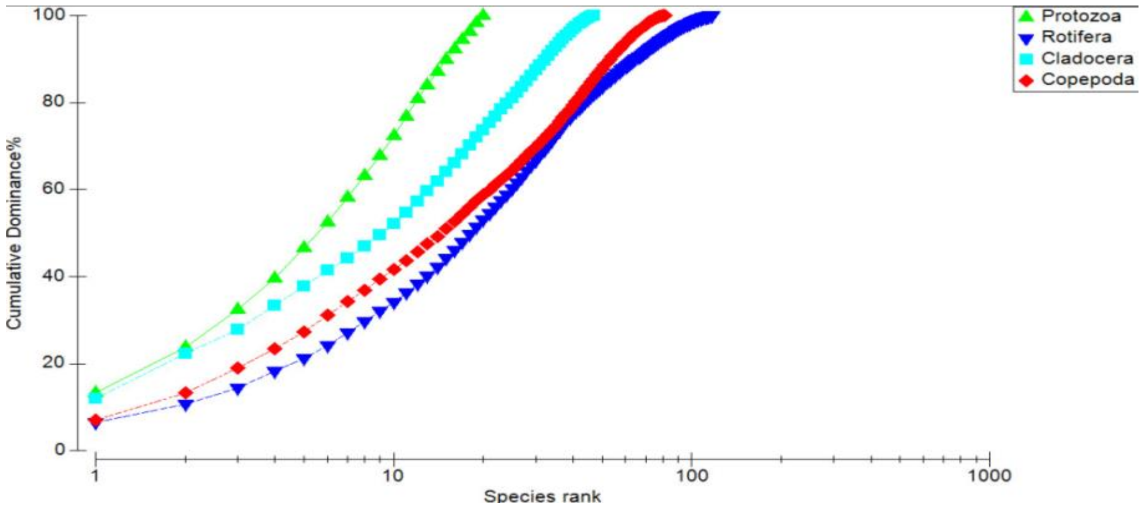

Fig. 6. Cumulative dominance (%) and species rank of 4 zooplankton groups in the Hau River

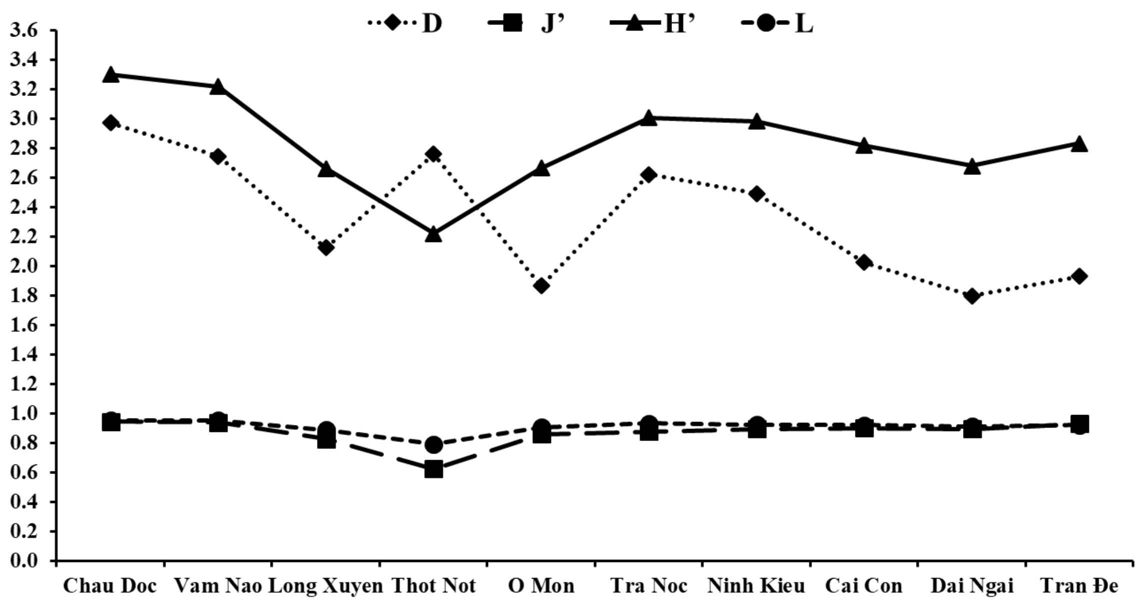

Fig. 7. Biological indicators of rotifer species along Hau River. Where: **H' =** Shannon-Wiener diversity index, **D =** Simpson's diversity index, **J' =** Margalef Richness Index, **L** = Lambda dominant species index

Table 4. Biological indicators of the zooplankton in the Hau River

Group	D (richness)	J' (similarity)	H'(loge) (diversity)	1-Lambda' (dominance)
Protozoa	1.8925	0.9455	2.8326	0.9320
Rotifera	8.3623	0.8888	4.2329	0.9793
Cladocera	4.0755	0.9028	3.4761	0.9551
Copepoda	6.5101	0.9091	3.9954	0.9737

5. Correlation of water quality and zooplankton

Twelve species with high frequency relatively high diversity, and dominance, (Supplemental Table 6) were used for the CCA analysis, and also the 30 sampling sites during 4 sampling periods. The analysis results have shown that temperature, clarity, water flow, phosphate and TSS values are very closely correlated with rotifer density at the sampling sites. The indicator such as pH, BOD_5, COD, TAN and chlorophyll-*a* are also closely correlated with the density of rotifers such as *B. rubens, B. plicatilis, B. angularis* and *M. bulla* (Fig. 8), and more specifical. The CCA analysis indicates that up to 64.8% of the total variability of the data recorded in the study can be explained.

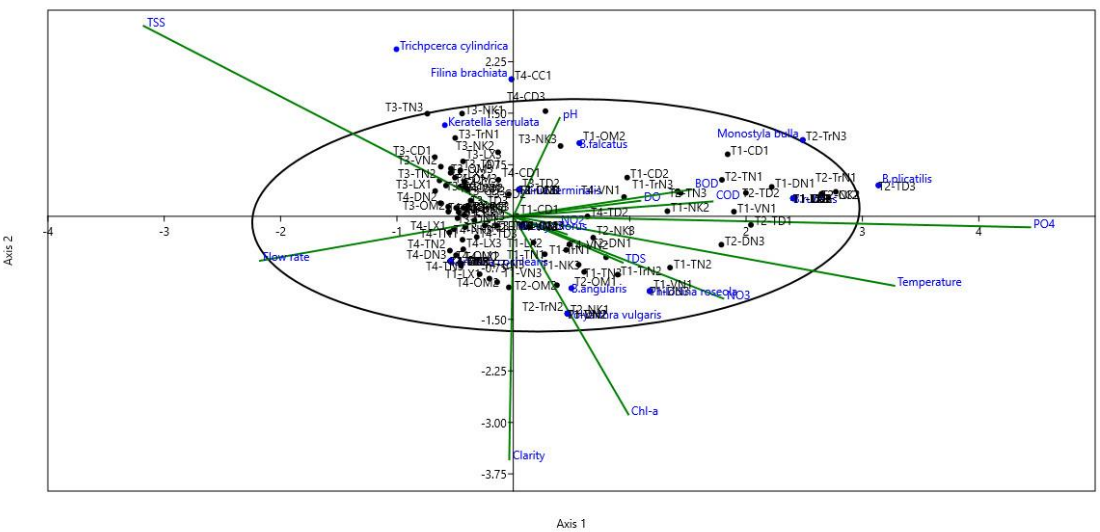

Fig. 8. Canonical correspondence analysis (CCA) analysis between water quality and rotifer density at every sampling site in the Hau River (Black dots; all the sampling locations along the Hau River, along with detailed codes, have been provided in Supplemental Table 1) together with the 13 species of rotifer: *B. calyciflorus*, *B. angularis*, *B. falcatus*, *B. plicatilis*, *B. rubens*, *F. brachiate*, *F. terminalis*, *K. serullata*, *P. roseola*, *P. vulgaris*, *T. cylindrical*, *M. bulla* (Blue dots) (Eigenvalue: 64.8%)

Supplemental Table 6. Biological indicators of some rotifer species in the Hau River

Rotifera	D (richness)	J' (similarity)	H'(loge) (diversity)	1-Lambda' (dominance)
B. calyciflorus	1.1848	1.0000	2.4849	0.9167
B. angularis	0.6861	1.0000	1.9459	0.8572
B. falcatus	1.7766	0.9096	2.6785	0.9005
B. plicatilis	1.1570	0.9782	2.4308	0.9067
B. rubens	2.2984	0.9227	3.0066	0.9371
F. brachiata	1.3581	0.9849	2.5993	0.9219
F. terminalis	2.9109	0.9508	3.2955	0.9543

K. serullata	3.0533	0.9237	3.3104	0.9532
P. roseola	0.8476	0.9417	2.0692	0.8572
P. vulgaris	2.0577	0.8972	2.8134	0.9154
T. cylindrica	1.3029	0.9469	2.4991	0.9062
M. bulla	0.2532	1.0000	1.0986	0.6669

DISCUSSION

In recent years, there has been an increased focus on studying the composition and distribution of rotifers in diverse aquatic environments across Vietnam **(Zhdanova, 2011; Dang, 2012; Nguyen *et al.*, 2020; Phan *et al.*, 2021)**. This study expanded on recent research focusing on understanding rotifer composition and distribution along Vietnam's Hau River. Moreover, the study incorporated water quality data and jointly analyzed the density of other zooplankton groups within the same river. Our research aligned the rotifer species observed here with those found in various regions of Vietnam **(Nguyen *et al.*, 2022)**. In addition to Vietnam, the rotifer species under study were compared to those detected in other tropical monsoon climate countries such as Thailand and Cambodia **(Meas & Sanoamuang, 2008; Ardrit *et al.*, 2013)**. We identified a total of 66 rotifer species, with *Brachionus*, *Keratella*, *Lecane*, *Monostyla*, *Ploesoma*, and *Filinia* being the most prevalent. Particularly, the presence of *Keratella*, *Lecane*, and *Brachionus* in freshwater bodies across Southeast Asia might be attributed to the consistent climate within the region.

Rotifers are an important zooplankton category, accounting for more than 50% of zooplankton production **(Herzig, 1987; Walz, 1995)**. This record is consistent with results found by **Zakaria *et al.* (2007)** and **Zorina-Sakharova *et al.* (2014)** that in low-salinity water, rotifers are dominated in zooplankton communities. Furthermore, **Bielanska-Grajner and Cudak (2014)** underlined that in low-salinity water habitats (less than 0.5), zooplankton diversity is dependent on rotifer species because freshwater or low saline intrusion is the best circumstance for rotifer populations to expand **(Bielańska-Grajner & Cudak, 2014)**. In the rainy season, the zooplankton species makeup in the lower Hau River resembled that of a freshwater or oligohaline community. However, as the dry season brought saline intrusion, elevating salinity beyond 5ppt, a notable transformation occurred in the zooplankton community structure along the stretch from Dai Ngai to Tran De locations. Consequently, there was an observed augmentation in the population densities of protozoa and copepods. On the other hand, a substantial decline was witnessed in the prevalence, encompassing the population density, of rotifers and cladocera as a consequence of this transition in salinity levels along this geographical gradient. A similar pattern of contrasting relationships with salinity for two distinct groups along the Hau River: protozoa and copepods showed a positive correlation, whereas rotifers and cladocera exhibited a negative correlation **(Zakaria *et al.*, 2007; Silva *et al.*, 2009; Špoljar *et al.*, 2018)**.

The observed rotifer species in the Hau River primarily belong to two groups: *Keratella* and *Brachionus*, aligning with findings from the study by **Bekelegen (2001)**. There was a difference in the upstream and downstream of the Hau River because of the salinity intrusions. In the Upstream, the dominant species are *P. vulgaris, B. rubens, B. falcatus, B. calyciflorus, F. terminalis, K. cochlearis, K. valga* and vice versa the downstream especially Dai Ngai and Tran De, the salinity rises during the dry season, and the rotifer species that thrive in high salinity become more prevalent in this location such as *Brachionus plicatilis, B. calyciflorus, B. rubens, B. caudatus, B. calyciflorus, Polyarthra vulgaris.* In regions characterized by distinct wet and dry seasons, salinity undergoes substantial annual fluctuations due to increased river discharge in the wet season, resulting in a decline in salinity. Conversely, in the dry season, inland seawater intrusion causes an increase in salinity **(Binh *et al.*, 2018; Nguyen *et al.*, 2020)**. The number of rotifer species recorded in the Hau River is higher in studies by **Nguyen *et al.* (2020)** and **Lien *et al.* (2022)**, with 53 species and 47 species, respectively. This difference may be due to differences in sampling area and sample collection time.

Based on the result in Fig. (1), concerning the sampling sites in brackish water, the composition of rotifera has a high degree of similarity and diversity compared to the sampling site in the freshwater. The sampling sites in freshwater areas have average similarity values and less diversity in rotifer composition. The results show that the sampling site has a significant impact on species composition and rotifer diversity along the Hau River during the sampling period. In Chau Doc and Vam Nao, areas with slow flow rates, particularly in the rainy season, were observed. These findings align with the study of **Jiang *et al.* (2023)**, which indicates substantial seasonal impacts on zooplankton diversity. Rotifers emerge as dominant during the wet seasons, attributed to their swift adaptability to environmental shifts and rapid life cycle completion **(Jiang *et al.*, 2023)**. Long Xuyen province demonstrates moderately lower rotifer density in the rainy season compared to the dry season, marking it as having the lowest rotifer density among upstream sites. In Thot Not province, the density of rotifera is the highest compared to other water bodies in the Hau River; during the rainy season, the rotifer density was recorded at 7,028±6,208 individuals/m^3, exhibiting an almost fourfold increase compared to 27,536±27,395 individuals/m^3 in the dry season. The high nutrients (Chlorophyll-a and phosphate) at Thot Not province help algae to grow, especially in the dry season when algae can grow better. Rotifers rely on algae as a primary food source, forming a crucial part of their diet **(Lubzens *et al.*, 1989)**, thus resulting in very high rotifer densities at this site. Chlorophylls are the main photosynthetic pigments in phytoplankton **(Roy *et al.*, 2011)**. The chlorophyll-a concentration increased in June, peaked during the summer, and began to decrease in September **(Neal *et al.*, 2006)**. At the Cai Con sampling site, the rotifer density isn't significantly higher than that observed at the Tran De site downstream, marking it as the area with the lowest rotifer density along the Hau River. Analysis indicates a notably low concentration of chlorophyll-a compared to other sites, directly impacting rotifer density. Moving to Dai Ngai, the average rotifer density is 7,039±6,789 inds/m^3. Several direct and indirect factors contribute to this growth in rotifer density, notably the concentrations of chlorophyll-*a*, TAN, nitrate and phosphate (14.8µ/ l, 0.763mg/ l, 0.339mg/ l and 0.509mg, respectively). These parameters define the trophic state, a vital aspect of aquatic ecosystems, significantly influencing zooplankton and rotifer community composition and fostering their growth **(Muñoz-Colmenares *et al.*, 2021; Phan *et al.*, 2021)**. Noteworthy rotifer species abundant at this sampling site include *B. plicatilis, K. serrulata, P. vulgaris* and *P. rosella*. The Tran De sampling site records one of the lowest rotifer densities among the Hau River sites, with slightly higher density observed during the rainy season compared to the dry season. This site's predominantly saline conditions throughout most of the year make it unsuitable for supporting rotifers, which typically thrive in freshwater environments. This factor contributes to the reduced rotifer density in these waters. Notably, *P. vulgaris* and *B. plicatilis* exhibit higher densities in this area.

At the Hau River sampling sites, rotifers are the group with the slowest increase in species rank, while protozoa show the highest cumulative increase in index. This difference suggests that salinity, temperature, and water flow have differential impacts on the relationship between cumulative dominance and species rank within zooplankton communities. These findings align with those of **Zakaria *et al.* (2007)**, who examined seasonal and salinity fluctuations in zooplankton populations. They found that protozoa and copepods consistently dominate, accounting for 51.19% and 27.9%, respectively, while rotifers represent a very low percentage (3.81%), with cladocerans even lower (0.26%) **(Zakaria *et al.*, 2007)**.

The CCA analysis reveals a strong correlation between several environmental factors and rotifer density at the sampling sites, notably including temperature, water clarity, flow rate, phosphate levels, and total suspended solids (TSS). Moreover, indicators like phosphate levels, temperature, total ammonia nitrogen (TAN), and chlorophyll-a also closely associate with the density of highly found rotifer species such as *B. rubens, B. plicatilis, B. angularis, and M. bulla.* Higher water phosphate levels promote increased algae abundance, serving as a food source for rotifers. This creates a positive correlation between rotifer distribution and phosphate concentration, linked to their dietary preferences **(Halabowski *et al.*, 2019)**. CCA analysis showed that water temperature, consistent with the study by **Vu and Huynh (2019)**, is positively correlated with the number of rotifers and the total phosphorus (TP) content in water, especially those of the Brachionidae family **(Vu & Huynh, 2019)**. A separate study suggested that specific rotifer species can enhance wastewater treatment by efficiently removing suspended particles. This is achieved through a dual mechanism: the consumption of particles by the rotifers and their positive influence on the settleability of wastewater particles, thereby improving clarity to some extent **(Lapinski & Tunnacliffe, 2003)**. The Hau River experiences a consistent tropical monsoon climate, with an average water temperature of 29.3 ± 0.9ºC, which supports optimal conditions for rotifers to thrive, and they reach their peak growth rates between 20 to 30°C **(Yoo *et al*., 2023)**. Furthermore, nutrients significantly impact rotifers indirectly through phytoplankton. Changes in nitrogen and phosphorus levels alter rotifer feeding habitats, affecting their community composition and density. Analysis across 10 locations in the Hau River revealed that areas with lower flow rates, such as Thot Not, have higher nutrient content (especially chlorophyll-a and PO_4^{3-}), resulting in increased abundance and diversity of rotifers. In contrast, regions like Tran De, affected by salinity year-round, experience lower nutrient content and higher flow rates, leading to decreased rotifer density, richness, and diversity in the water body.

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