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Original Article

Seasonal Variations in Ostracod Community Structure and Physicochemical Parameters in Khanki Headworks, Pakistan

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ABSTRACT

Protozoans, rotifers, ostracods and copepods are principal groups of zooplanktons. These organisms are crucial biotic elements, and impact all functional features of aquatic ecosystems. **Objective:** To assess the impacts of environmental variables on the abundance, biodiversity and seasonal distribution of ostracods in Khanki Headworks, Pakistan. To evaluate the contribution of ostracods as bio-monitors of contamination in freshwater ecosystems. **Methods:** Month wise separate water samples were taken for different environmental variables from four sampling sites (each with 3 sub-sites), for study period of one year (February 2021 to January 2022), whereas, samples of microcrustaceans (ostracods) were collected with planktonic net (mesh size: 37µm). **Results:** In total, 5 species of ostracods, associated to 4 genera and 2 families were identified from February 2021 to January 2022. Heterocypris incongruence was the most abundant species, whereas, Dolerocypris sinensis was noted as least abundant species. Ostracods abundance and biodiversity were monitored highest in June and minimum in the month of January. Results of Pearson correlation showed that physicochemical parameters, electrical conductivity (EC), temperature, turbidity, total dissolved solids (TDS) and pH have positive effects on the density and diversity of ostracods. In contrast, dissolved oxygen (DO) showed negative relationship with the abundance and diversity of planktonic ostracods. These findings were further validated by Canonical correspondence analysis (CCA). **Conclusions:** This investigation revealed that physicochemical parameters generally govern the population dynamics of ostracods.

INTRODUCTION

Ostracods are tiny bivalved microcrustaceans inhabiting different water bodies [1, 2]. There are approximately 65,000 known species (living and fossil) of ostracods but only 2340 species are freshwater [3]. Their calcified carapaces are species specific and they are used in paleoecological and paleobiological studies [4, 5]. Ostracod populations are usually parthenogenetic and bisexual, whereas, few are mixed populations [6]. They can be located in all types of aquatic ecosystems. Ostracods generally inhabit, benthic, nektonic and interstitial habitats [7-9]. Nektonic species can change location by swimming and avoid the predators, while benthonic species move on the surface of sediments by crawling [10]. Vectors (e.g., human, insects, fishes and birds) are involved in their distribution over long distances. Ostracods perform a very prominent role as an intermediate link in different food webs by carrying energy and primary production from lower to upper trophic levels. Many freshwater ostracods exhibit seasonal variations in their occurrences [11]. If patterns of species occurrences within a certain time

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period of seasons are well documented, this knowledge can be extremely helpful for interpreting ecological attributes of that area. Hydrographical parameters play a crucial role in regulating the community structure of aquatic invertebrates [12]. Microcrustaceans (ostracods) are very sensitive to slight environmental changes and exhibit different preferences and species-specific responses to various environmental variables [13]. Water quality of aquatic ecosystems is declining day by day due to anthropogenic interference and industrial growth. Hence, providing ecological data about such types of species can be exploited for assessment of environmental conditions of different artificial and natural habitats by means of considering ostracods as indicator. Ostracods are very good bioindicator species of pollution and water quality [14, 15]. River Chenab is well known freshwater ecosystem in Pakistan and it has very diverse and rich aquatic flora and fauna [16, 17]. Khanki Headworks is very prominent site, providing habitat to distinguished fish fauna, however, no previous effort is reported in this area regarding our present investigation. Present study is the first baseline work that was conducted for a period of one year (February 2021 to January 2022) in Khanki Headworks.

METHODS

Khanki Headworks (32°24'11" N, 73°58'19" E) is one of the major headworks that are situated on Chenab River, Punjab, Pakistan. It plays its part in prevention of floods and irrigation of barren agricultural fields (three million acres). It is a diversion headworks that diverts water to the Lower Chenab Canal and fifty-nine minor tributaries. Apart from geostrategic importance this area is known for fishing due to its diverse and rich fish fauna. For the analysis of environmental parameters separate monthly water samples from four designated sites were assembled for a period of 12 months (February 2021 to January 2022). For sampling pre-cleaned plastic bottles (1 liter) were utilized. Water sampling was usually performed in early hours of the day (9.0 A.M to 12 P.M). Measurements of Temperature, turbidity, pH, total dissolved solids (TDS), electrical conductivity (EC) and dissolved oxygen (DO) were noted in the field immediately by using their respective meters [18, 19]. Other hydrological variables such as total hardness (TH) and total alkalinity (TA) were examined in laboratory by following standard guidelines [20]. For one year (February 2021 to January 2022) ostracod samples were taken month wise from four specific locations (each with 3 sub-sites) by using planktonic net (mesh size: 37µm). Samples were collected from water by placing Planktonic net in horizontal position and passing 60 liters of water through it. Pre-cleaned plastic bottles (50 ml) were used for microcrustacean sampling. After that formalin solution (5%) was added in bottles for fixation and further analysis [21, 22]. Ostracod species were identified with relevant literature and standard keys [23-29]. Identification of ostracods was made possible by observing their carapace structure and delicate body parts. Enumeration of ostracods was performed by Sedgewick-Rafter counting chamber [30]. Microcrustaceans (ostracods) were observed under an inverted microscope (LEICA HC 50/50) fitted with 5 mega pixel camera and Photographs of ostracods were also taken. Living ostracod samples stained with neutral red (1%) were also examined under the microscope. Relative abundance and seasonal diversity of ostracods were computed with Shannon-Weaver (H) and Simpson (D) diversity indices [31, 32]. Species richness (SR) was formulated by Margalef et al., whereas species evenness was determined by following Pielou formula [33, 34]. Relationships between ostracod species and different hydro-geographical parameters were estimated by Pearson's correlation. R software was utilized to execute One way ANOVA, to assess marked difference among ostracod population density in various months. Pearson's correlation was also performed using R software. Relationships between ostracod species and various months were evaluated by Principal Component Analysis (PCA), whereas, correlations between crustacean (ostracod) fauna and different environmental variables were examined by using CCA (Canonical Correspondence Analysis). XL stat (2022) was employed for PCA and CCA.

RESULTS

We identified 5 species of ostracods. Ostracod species belonged to 4 genera and 2 families (Table 1).

Table 1: List of ostracods species identified from Khanki Headworks (February 2021 to January 2022)

Sr. No.	Family	Genus	Species
1	Cyprididae	Heterocypris	Heterocypris incongruence
2			Heterocypris punctata
3		Cypridopsis	Cypridopsis okeechobei
4		Dolerocypris	Dolerocypris sinensis
5	Darwinulidae	Darwinula	Darwinula stevensoni

Heterocypris incongruence was observed as most prevalent ostracod species, whereas, Dolerocypris sinensis was least abundant species (Figure 1).

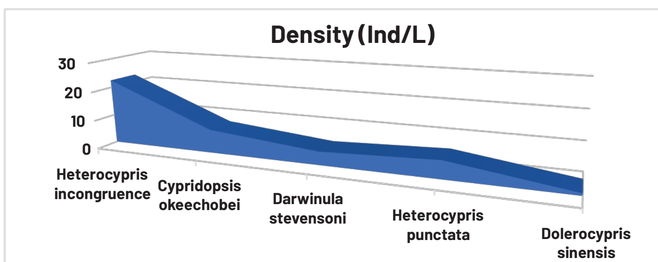

Figure 1: Relative density (Ind/L) of ostracod species assembled

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from Khanki Headworks

Hetrerocypris (2 species) was noted as the most diverse and abundant (14.04±5.17) ostracod genus (Figure 2).

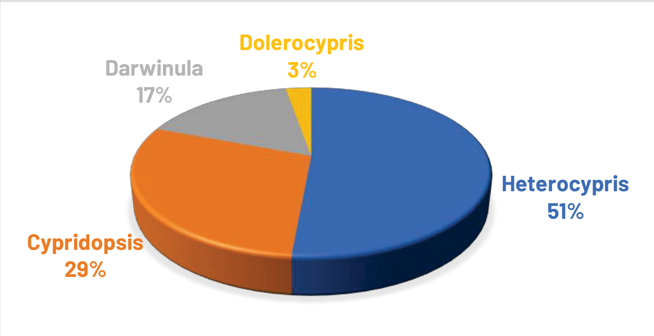

Figure 2: Percentage representation of ostracod genera identified from Khanki Headworks (February 2021 to January 2022)

Shannon-Weaver diversity index (H) manifested highest values for Ostracoda in June and least values in January. Similar trend was recorded by Simpson diversity index and highest values (0.71) for ostracods were observed in June, while lowest values (0) were noted in the month of January (Figure 3).

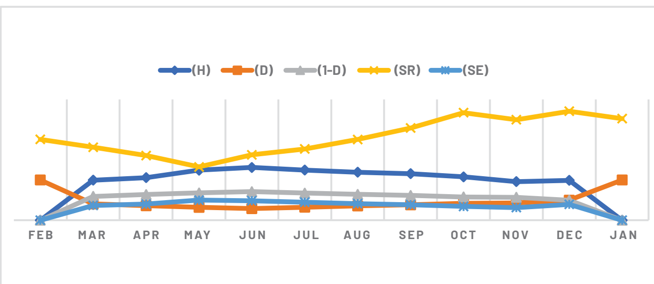

Figure 3: Month wise fluctuations of ostracods diversity indices from Head Khanki (February 2021 to January 2022)

D (Simpson index of dominance), H (Shannon-weaver diversity index), 1-D (Simpson index of diversity), SE (Species evenness), SR (Species richness)

Maximum biodiversity of ostracods (4 species) was explored in June, whereas, in January, minimum diversity of ostracods (1 species) was recorded. Statistically significant difference in ostracod density from February 2021 to January 2022 was computed by ANOVA (Table 2).

Table 2: Analysis of variance of ostracods (P<0.05) from Khanki Headworks (February 2021 to January 2022)

Source of Variation	SS	DF	MS	F	p-value	F crit
Between Groups	54.752	1	54.752	7.496	0.012	4.300
Within Groups	160.682	22	7.303	-	-	-
Total	215.434	23	-	-	-	-

SS= Sum of square, DF= Degree of freedom, MS= Mean of square, P= Probability, F= f-Distribution

Based on four principal components, 98.14% of total variance was reflected by PCA. In total 78.68% variance was displayed by axis F1 (47.55%) and axis F2 (31.13%), in ostracod community structure (Figure 4).

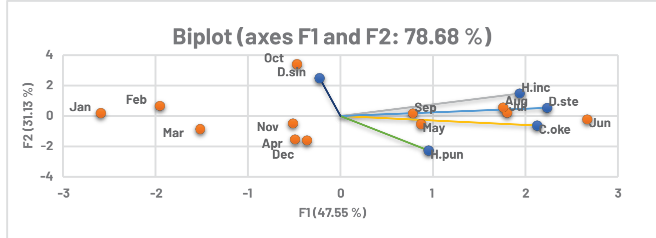

Figure 4: Principal component analysis (PCA), graph of 5 ostracod species assembled from Head Khanki

CCA symmetric map exhibited the impacts of physicochemical variables on the population dynamics of planktonic ostracods during one year study duration (Figure 5). The first axis (53.72%) and second axis (26.74%) of CCA depicted 80.46% of the trended information for ostracod abundance (5 species).

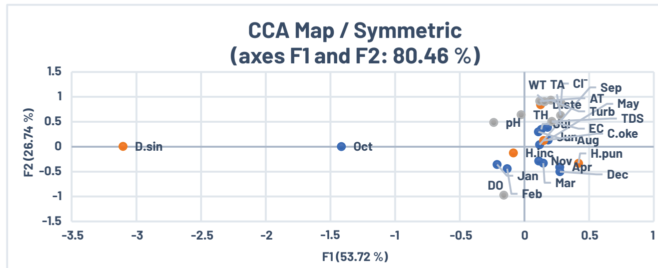

Figure 5: Ordination of 5 ostracods species on CCA triplot, reflecting the impacts of 10 environmental variables in Khanki Headworks

In present analysis temperature values fluctuated between 39°C to 16°C, being highest in June and minimum in January. It was found that temperature positively influenced the population abundance of microcrustacean ostracods. Ostracods exhibited conspicuous growth in summer months (May, June, July) with maximum biodiversity (4 species) and abundance (5.5±1.86) were recorded in June, whereas, minimum population density (1.25±0.29) and diversity (1 species) were observed in January (Figure 6).

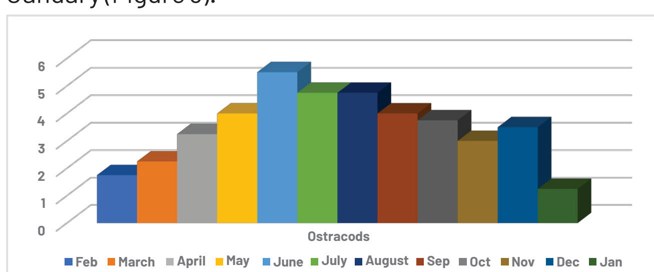

Figure 6: Population density of ostracods month wise from Khanki Headworks

Relatively alkaline values of water pH (7-7.9) were noted during study period, being maximum (7.9) in July and minimum (7) in January (Figure 7).

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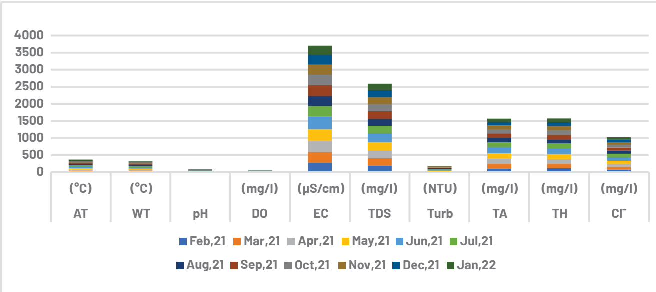

Figure 7: Seasonal fluctuations of 10s environmental variables Temperature (°C), Dissolved oxygen (mg/L), pH, Total hardness (mg/L), Electrical conductivity (µs/cm), Turbidity (NTU), Total dissolved solids (mg/L), Total alkalinity (mg/L), Chlorides (mg/L) in Khanki Headworks.

Water pH also displayed positive interaction with ostracods. DO values fluctuated between 5.34-6.97 in different months. Maximum value (6.97) was noticed in January, whereas, least value (5.34) was measured in June. According to Pearson's correlation dissolved oxygen (DO) displayed negative correlation with microcrustacean (ostracods) density and biodiversity. Electrical conductivity (EC) was reported maximum (369 µs/cm) in June and lowest (270 µs/cm) in January. Total hardness (TH) was observed highest (170 mg/l) in June and its least value (110mg/l) was measured in January. EC and TH were explored to have positive relationship with crustacean (ostracod) fauna. Turbidity escalated in summer months, whereas, it reduced in winter months. Maximum value (258 mg/l) of total dissolved solids (TDS) was documented in June while its lowest value (189 mg/l) was noted in January. Water alkalinity was monitored maximum (180 mg/l) in June and lowest (100 mg/l) in January. Population attributes of ostracods were observed to be positively affected by these above mentioned (Alkalinity, Turbidity, TDS) environmental variables (Table 3).

Table 3: Pearson correlations between ostracods and physicochemical parameters at Khanki Headworks (February 2021 to January 2022)

	Ostra-cods	AT	WT	pH	DO	EC	TDS	Turb	TA	TH	Cl⁻
Ostra-cods	1	-	-	-	-	-	-	-	-	-	-
AT	0.895	1	-	-	-	-	-	-	-	-	-
WT	0.887	0.994	1	-	-	-	-	-	-	-	-
pH	0.373	0.406	0.409	1	-	-	-	-	-	-	-
DO	-0.905	-0.956	-0.953	-0.486	1	-	-	-	-	-	-
EC	0.629	0.780	0.755	-0.136	-0.645	1	-	-	-	-	-
TDS	0.631	0.783	0.758	-0.133	-0.649	0.999	1	-	-	-	-
Turb	0.882	0.899	0.897	0.527	-0.939	0.537	0.539	1	-	-	-
TA	0.640	0.835	0.819	-0.022	-0.737	0.943	0.945	0.609	1	-	-
TH	0.658	0.821	0.789	0.021	-0.699	0.931	0.931	0.616	0.912	1	-
Cl⁻	0.863	0.957	0.950	0.298	-0.935	0.766	0.768	0.897	0.866	0.813	1

DISCUSSION

The purpose of this study was to investigate the seasonal shifts in community structure of planktonic microcrustacean (Ostracoda). After qualitative analysis of samples, 5 ostracod species were documented. Heterocypris incongruence was the most common ostracod species that was observed in all sampling months. It is actually a cosmopolitan species labelled as "horse-trough ostracod" [35]. According to quantitative analysis, Heterocypris (51.45%) was the most abundant ostracod genus. In summer months, ostracods showed an appreciable rise in density and diversity, whereas, in winter season minimum abundance of ostracods was recorded. It was discovered that high temperature encourages the growth of most ostracod species [36]. Maximum diversity of ostracods (4 species) was noticed in the month of June, whereas, minimum diversity was recorded in January (1 species). Photosynthetic activity also accelerates as temperature rises which promotes the microcrustacean abundance [21]. According to our findings pH values were relatively alkaline during sampling months [37, 22]. pH mounted during summer season because of eutrophication and elevated levels of nitrates ($NO3^-$) and carbonates ($CO32^-$) in water, as reported previously [38]. Biodiversity and population abundance of ostracods flourished at higher pH [39]. Elevated DO levels were noted during winter season, whereas, DO values went down in summer season and lowest values were documented in June (5.34 mg/l). During summer months BOD (biological oxygen demand) rises considerably for decomposition of organic matter that brings about low DO levels in water [40]. Rising temperature can limit the O2 solubility in water [18, 21]. However, dissolved oxygen level was never reported below 5 mg/l that is regarded as threshold level for survival aquatic life [41-43]. A negative correlation was recorded between ostracods abundance and DO level [44, 21]. In summer, elevated values of total dissolved solids (TDS) were noticed because high temperature expedites the decaying process of vegetation that causes more addition of TDS in water [45]. Rising values of electrical conductivity (EC) were noted in summer season and lower values were recorded in colder months [46]. EC values fluctuated between 270 µS/cm to 369 µS/cm. Improved evaporation rate might be the cause of elevated EC values in summer season. Both variables (EC and TDS) positively influenced the density of ostracods. High turbidity was observed especially in rainy season (July and August). During monsoon heavy precipitation disrupts the settled bottom sediments that brings about a surge in water turbidity [47]. However, it facilitates the ostracod abundance because of further food availability [48]. High values of total alkalinity (TA) and total hardness (TH) were recorded in summer months and lower values in winter. For the estimation of hardness, generally magnesium (Mg++) and

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calcium (Ca2+) ions are examined, whereas, for the estimation of TA carbonates (CO32-) and bicarbonates (HCO3⁻) are usually considered [17]. Both parameters (TH and TA) were noticed to exert positive impact on population density of microcrustaceans [49]. Shannon-Weaver index of diversity (H), computed highest ostracod biodiversity in June and minimum in the month of January. Values of Simpson diversity index for ostracods varied between 0 to 0.71, being highest (0.71) in June and lowest (0) in January. Simpson index of dominance computed minimum value for ostracods (0.29) in June while maximum value (1) in January. PCA findings displayed 2 species in group 1 (Upper right), 2 species in group 2 (bottom right), not a single species in group 3 (bottom left) and 1 ostracod species in group 4 (upper left). Maximum ostracod biodiversity was associated to the summer months of (May, June, July, August). CCA results manifested interrelationships between planktonic ostracods and environmental variables. CCA symmetric map represented that different environmental parameter (temperature, pH, TDS, TH, TA) exert pronounced effects on the distribution and abundance of ostracods.

CONCLUSIONS

Present study contributed to enhance baseline knowledge, data and information about seasonal variations in ostracod species distribution in relation to environmental variables in Khanki Headworks. Physicochemical variables (temperature, EC, TH, DO, TDS) regulated the community structure of ostracods. In summer months, population density of ostracods increased significantly. Similarly, diversity of ostracods also improved during summer season, whereas, ostracod population abundance and diversity decreased in winter months. Our findings indicated that ostracods are highly sensitive to slight changes in physical and chemical environment. Thus, they can provide essential information and facts regarding the trophic status of freshwater ecosystems.

Authors Contribution

Conceptualization: MAR, NR
Methodology: MAR
Formal analysis: MAR, HA
Writing-review and editing: MAR
All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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