Original Article

Biodiversity, distributions and isolation of microplastics pollution in finfish species in the Panjkora River at Lower and Upper Dir districts of Khyber Pakhtunkhwa province of Pakistan

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Abstract

The basic aim of this study was aimed to determine the ichthyofaunal diversity of River Panjkora in both upper and lower Dir districts in Khyber Pakhtunkhwa province of Pakistan. Fish samples were collected by using fishnets from March to September 2020. A total of 724 specimens were collected and classified into 5 families, 14 genera, and 18 species. The overall results revealed that most fish fauna of river Panjkora contains 8 species of family Cyprinidae (56.49%) followed by 4 species of Nemacheilidae (24.44%), 2 species of Channidae (10.63%), and Sisoridae (7.04%), and 1 species of Mastacembelidae (1.38%). Among all kinds of fish species, Schizothorax plagiostomus (16.57%) was highly dominated and followed by Carassius auratus (11.87%) and Racoma labiata (9.66%) and were reported as highly abundant, especially during April, May, and June. The least abundant species were Glyptothorax punjabensis, Glyptothorax sufii, and Mastacembelus armatus, that constituting 2.48%, 2.20%, and 1.38%, respectively, of the total fish samples. The Overall Simpson’s diversity (1-D= 0.919) and Simpson’s Reciprocal index values (1/D= 12.3876), and Shannon’s index (H= 2.68) were indicating that river Panjkora contains a quite rich and diverse group of fish species. The highest microplastics observed in site 7 compared to other study area. Conservation steps should be taken as a top priority to protect and conserve the marine environment and natural heritage from further loss, extinction and stop or minimize losses incurred through irresponsible fishery practices.

Keywords: Ichthyofaunal diversity, distribution of fish species, microplastic pollution, Panjkora river.

Resumo

O objetivo básico deste estudo foi determinar a diversidade ictiofaunística do rio Panjkora nos distritos de Lower e Upper Dir, na província de Khyber Pakhtunkhwa, no Paquistão. Amostras de peixes foram coletadas com redes de arrasto de março a setembro de 2020. Foram coletados 724 espécimes, classificados em 5 famílias, 14 gêneros e 18 espécies. Os resultados gerais revelaram que a maioria da ictiofauna do rio Panjkora contém 8 espécies da família Cyprinidae (56,49%), seguidas por 4 espécies de Nemacheilidae (24,44%), 2 espécies de Channidae (10,63%) e Sisoridae (7,04%), e 1 espécie de Mastacembelidae (1,38%). Entre todas as espécies de peixes, Schizothorax plagiostomus (16,57%) foi altamente predominante, seguida por Carassius auratus (11,87%) e Racoma labiata (9,66%), e elas foram relatadas como altamente abundantes, especialmente nos meses de abril, maio e junho. As espécies menos abundantes foram Glyptothorax punjabensis, Glyptothorax sufii e Mastacembelus armatus, que constituíram 2,48%, 2,20% e 1,38%, respectivamente, do total de peixes amostrados. O índice de diversidade de
1. Introduction

One billion people globally consume fish, which is one of the major sources of high-quality protein and are used to develop nation economy (Khalid et al., 2020; Ahmad et al., 2020). Fish is the cheapest source of high-quality protein fatty acids and antioxidants that can protect our body against certain diseases like cancer. Fish are susceptible to ecological modifications, and their diversity in a broad spectrum of differential tolerance reflects the health status of the ecosystem (Chowdhury et al., 2019; Hassan et al., 2020a; Sabbir et al., 2020; Hussain et al., 2021). Changes in ecosystems of the food tropical level implanted remarkable effects on the abundance and distribution of fish. Pakistan has rich freshwater fish fauna, which is represented by 193 fish species (Rafique and Khan, 2012; Hassan et al., 2023). More than 180 species had been reported in the Indus River in Pakistan (Mirza and Mirza, 2014). Many researchers had made valuable contributions in the study of the fish fauna of the Khyber Pakhtunkhwa, including Ahmad and Mirza (1963), Khan (1983), Butt (1986), Javed et al. (1996), Mirza (2007), Ahmad and Hasan (2011), Hasan et al. (2013, 2015), Saeed et al. (2013), Ishaq et al. (2014), Muhammad et al. (2014) and Akhtar et al. (2014), respectively. However, the broad range of biodiversity and distribution patterns of fishes found in the Panjkora river, and its tributaries require more investigation. Fish fauna of the Panjkora river contains a high proportion of endemic species were belong to three genera includes i.e., Triplophysa, Schistura, and Glyphiathorax. All these species are mainly cold water fishes and confined only in headwater hilly streams and rivers, and isolated from warm water fish populations stretches between them. The growing decline in fish biodiversity has now shocked the world conservation community. Roughly about 20% of the world’s freshwater fishes are currently in endangered or extinct stages (IUCN, 1989; Hassan et al., 2020b). While the identification and characterization of existing species had been considered as an essential part of their conservation and needs more attention in the scientific literature (Hassan et al., 2020a; Romanova et al., 2018). Fish species composition and their relative abundance might be changed due to the seasonal variations (Jisr et al., 2018; Abro et al., 2020; Islam et al., 2020). The present study was aimed to know the current status of the fish fauna of River Panjkora at Districts Dir (Upper and Lower), Khyber Pakhtunkhwa, Pakistan.

2. Materials and Methods

2.1. Ethical statement

This study was carried out with the strict commendations and approval of the Ethical Committee for Experiments on Fisheries of the University of Karachi and University of Malakand KPK Pakistan.

2.2. Study area

District Dir is located in the Khyber Pakhtunkhwa province of Pakistan, at 34º, 37′ to 35º, 07′ North and 71º, 31′ to 72º, 14′ East, and found at 823 meters (2700 feet) above the sea level. Kohistan of Upper Dir is the actual origin of River Panjkora. This river flows southward and almost passes through the middle of both the Lower and Upper Dir districts, and meets the Swat River in Sharbatti, District Malakand, behind Totakan (Bosaat Bridge). The Panjkora River was called for its five major tributaries, which meet it at four locations: Chukitan for Barawal Stream, Sheringal for Gwaldi Stream, Akhagram for Dobando Stream, and both Nurhund and Usherai Dara Streams for Nurhund Stream (Figure 1).

2.3. Fish collection

In this study, collection of fish sampling were made at seven locations in River Panjkora during the study period from March to September 2020 (Figure 1). Fishes were caught with gill nets, cast nets and hooks, and preserved in 10% formalin solution.

2.4. Measurements and weighting

All measurements and weight of fishes were made by using the meter scale and Venire calliper. The total length (TL) of fishes was resolute by using scale and china tape. Body weight was noted with the help of an electric balance. Lateral-line scales were counted from the anterior to the posterior region scales. Transversal scales were counted as scale rows found above the lateral line and below the lateral line separately.

2.5. Fish identification

All fishes identified were made by using standard taxonomic keys provided by Mirza (1990) namely “Pakistan ki Taza Pani ki Machliyan”, Freshwater Fishes of the Indian Region (Jayaram, 1999) and Inland fishes of India and adjacent countries by Talwar and Jhingran (1991) (Figure 2).

2.6. Microplastic isolation

Microplastic isolated from the selected fishes by following the methods of Hassan et al. (2023).

3. Results and Discussion

A total of 724 specimens contain 5 families and 18 species as shown in the Table 1. Cyprinidae was the most dominant 56.49% (n=409/727) followed by...
Figure 1. River Panjkora District Dir (Upper and Lower) Pakistan digits refer to the sampled localities (see Table 1).

Figure 2. Fish diversity in river Panjkora at Districts Dir Upper and Lower, Khyber Pakhtunkhwa, Pakistan.
Table 1. List of fish species with their sampling locations and geographic specification.

| Stations       | S. No | Location               | Species                                                                 |
|----------------|-------|------------------------|-------------------------------------------------------------------------|
| Upper Dir district | 1     | Dobando stream         | Schizothorax plagiostomus, Schizothorax esocinus, Acanthocobitis botia, Carassius auratus, Gara gotyla |
|                | 2     | Usherai stream         | Schizothorax plagiostomus, Schizothorax esocinus, Triplophysa microps, Acanthocobitis botia, Carassius auratus, Gara gotyla |
|                | 3     | Wari                   | Tor putitora, Schizothorax plagiostomus, Schizothorax esocinus, Triplophysa microps, Acanthocobitis botia, Carassius auratus, Gara gotyla |
| Lower Dir district | 4     | Khall                  | Schizothorax plagiostomus, Schizothorax esocinus, Carassius auratus, Gara gotyla |
|                | 5     | Ranai                  | Schizothorax plagiostomus, Schizothorax esocinus, Carassius auratus, Gara gotyla |
|                | 6     | Asharighat and Konhaye stream | Schizothorax plagiostomus, Schizothorax esocinus, Carassius auratus, Gara gotyla, Glyptothorax sufii, Glyptothorax punjabensis, Channa gachua, Channa punctata, Barilius vagra, Puntius ticto, Schistura alepidota |
|                | 7     | Timergara bypass and Rhound stream | Schizothorax plagiostomus, Racoma labiata, Carassius auratus, Gara gotyla, Channa gachua, Channa punctata, Mastacembelus armatus, Tor putitora |

nemacheilidae 24.44% (n=177/724). Other families were represented to a lesser extent sisoridae 10.63% (n=77/724) and channidae 7.04% (n=51/724). The least dominant family was mastacembelidae 1.38% (n=10/724). Family cyprinidae was represented by eight species (Schizothorax plagiostomus, Schizothorax esocinus, Racoma labiata, Tor putitora, Barilius vagra, Garra gotyla, Puntius ticto and Carassius auratus) with high diversity index value (D= 0.057396 and H= 1.37756). Out of these eight species the most dominant species was Schizothorax plagiostomus with 16.57%, followed by Carassius auratus with 11.8% and Racoma labiata with 9.66%. Schizothorax plagiostomus was the top one distributed in all the sites studied. While family Nemacheilidae was the second dominant family with high diversity value (D= 0.0162 and H= 0.66982), and includes depiction species i.e., Schistura alepidota, Schistura prashari, Triplophysa microps and Acanthocobitis botia.

The most abundant species of family Megachilidae was Schistura alepidota 8.28% and found in one site. Family sisoridae was represented by three fish species, Glyptothorax punjabensis, Glyptothorax sufii and Nangra robusta with diversity value (D=0.004493 and H=0.3438). While the most abundant species of family Sisoridae was Nangra robusta having 5.93% of the total catch and distributed in three sites. Family Channidae includes two fish species, i.e., Channa gachua and Channa punctata that contributed 7.04% of total fish caught and with total diversity value (D=0.002464 and H=0.2346). The most abundant species of family channidae was Channa gachua having 4.14% of the total catch and was found in two zones. Family Mastacembelidae was represented by only single species i.e., Mastacembelus armatus that contributed 1.38% of the total fish caught and having total diversity value (D=0.0001719 and H=0.05915) and found in only one side of the study area.

Fishes caught during this study were showing their abundance in the month of May with highest diversity value (D= 0.323777 and H=0.94565), and less abundant in September with lowest diversity value (D= 0.022855 and H=0.06675). The overall Simpson's diversity (1-D= 0.919) and Simpson's Reciprocal index values (1/D= 12.3876), and (Shannon's index = 2.68) were indicating that River Panjkora is quite rich and contain diverse groups of fish species. Thus, this study also revealed that fish diversity of River Panjkora was high in the month of May with 14 fish species and 35% abundance, followed by June with 11 fish species and 19% abundance, July with 12 fish species and 15% abundance, April with 10 fish species and 12% abundance, March with 6 fish species and 7% abundance, and August and September months were with least diversity (6 to 8 fish species and 7% and 2% abundance as shown in the Table 2 and 3, respectively. In this study, about 5 families, 14 genera and 18 fish species of the and were reported in Panjkora River, which was less than Islam and Siddiqi (1971), who also had been reported eight families, 16 genera and 21 fish species from river Jhelum. On the other hand, Mirza and Sandhu (2007) had described about 189 species from the Indus River; while Rafique et al. (2003) had identified 13 families and 50 fish species of 13 families from Jinnah Barrage at Indus River. Abro et al. (2020) had reported about 44 fish species from Indus River. Mirza and Mirza (2014) had described 51 species belongs to 39 genera from River Jhelum Pakistan. Hasan et al. (2013) had reported about 50 species from River Swat, while Iqbal et al. (2013) had found 60 fish species from the Attok region of Pakistan. Mirza and Bhatti (1999) had also reported 82 fish species from Chenab River, and Rafique and Khan (2012) identified 193 fish species from fresh water reservoirs of Pakistan and their biodiversity were higher than the present study. By comparing the present study with studies of other researchers such as, Ullah et al. (2014) who reported 13 fish species from river Panjkora, Ullah et al. (2014) also reported 11 fish species from Konhaye Stream of district Lower Dir of Khyber Pakhtunkhwa. Hasan et al. (2015) had also observed 25 fish species from river Panjkora, of Lower Dir. Akhtar et al. (2014) had been worked on fish fauna of river Arunai, Matta, and Swat of KPK province of Pakistan.
and also reported 20 species, which were belonging four families and three orders. Akhtar et al. (2014) also described 18 fish species belonging to the three families and three orders from rivers Swat of Khyber Pakhtunkhwa province of Pakistan. Hasan et al. (2015) had also reported about 22 fish species belongs to 10 families from lentic water bodies of Indus River at Ghazi Ghat of Pakistan. Ali et al. (2010) identified 15 fish species were belonging to the 13 genera and 5 families from Suleman Mountain Range of D.G. Khan. Hasan et al. (2013) had reported 16 fish species belongs to Four orders, 12 genera, and 5 families from Bajour Agency. Akhtar et al. (2014) had reported 10 fish species, and also a new record *Cyprinus carpio* from river Barandu in district Buner of KPK, Pakistan. Ali et al. (2010)

Table 2. List of fish species identified from River Panjkora with their Distributional status and Diversity Index

| Order               | Family                              | Species                | Distribution status | % of Abundance | Simpson's index (D) Pi2 ×X | Shannon's index (H) Pi (log Pi)>S |
|---------------------|-------------------------------------|------------------------|--------------------|----------------|-----------------------------|---------------------------------|
| Cypriniformes       | Cyprinidae (number of species, 8)   | *Schizothorax* plagiotomus | Indigenous         | 16.574 | 0.1657 | 0.02728 | 0.29789 |
|                     |                                     | *Schizothorax* esocinus  | Indigenous         | 4.143  | 0.0414 | 0.00166 | 0.13192 |
|                     |                                     | *Racoma labiata*        | Indigenous         | 9.668  | 0.0966 | 0.00922 | 0.22588 |
|                     |                                     | *Tor Putitora*          | Indigenous         | 3.314  | 0.0331 | 0.00105 | 0.11293 |
|                     |                                     | *Barilius vagra*        | Indigenous         | 2.071  | 0.0207 | 0.00040 | 0.08032 |
|                     |                                     | *Garra gotyla*          | Indigenous         | 4.696  | 0.0469 | 0.00214 | 0.14363 |
|                     |                                     | *Puntius ticto*         | Endemic            | 4.143  | 0.0414 | 0.00166 | 0.13192 |
|                     |                                     | *Carassius auratus*     | Exotic             | 11.878 | 0.1187 | 0.01396 | 0.25306 |
| Nemacheilidae (Number of species, 4) | | *Schistura alepidota* | Endemic            | 8.287  | 0.0828 | 0.00676 | 0.20639 |
|                     |                                     | *Schistura prashari*    | Endemic            | 7.734  | 0.0773 | 0.00588 | 0.19797 |
|                     |                                     | *Triplophysa microps*   | Endemic            | 4.972  | 0.0497 | 0.00240 | 0.14923 |
|                     |                                     | *Acanthocobitis botia*  | Endemic            | 3.453  | 0.0345 | 0.00114 | 0.11623 |
| Siluriformes        | Sisoridae (Number of species, 3)    | *Glyptothorax panjabensis* | Endemic            | 2.486  | 0.0248 | 0.00058 | 0.09185 |
|                     |                                     | *Glyptothorax sufi*     | Endemic            | 2.209  | 0.0220 | 0.00045 | 0.08425 |
|                     |                                     | *Nangra robusta*        | Endemic            | 5.939  | 0.0593 | 0.00345 | 0.1677 |
|                     |                                     | *Channa gachua*         | Indigenous         | 4.143  | 0.0414 | 0.00166 | 0.13192 |
|                     |                                     | *Channa punctata*       | Indigenous         | 2.900  | 0.0290 | 0.00080 | 0.10269 |
| Channiformes        | Channidae (Number of species, 2)    | *Mastacembelus armatus* | Indigenous         | 1.381  | 0.0138 | 0.00017 | 0.05915 |
| Mastacembeliforms   | Mastacembelidae (Number of species, 1) | | | Total | 100 | 1 | D = 0.08072 | H = 2.68492 |

Simpson’s Diversity index (1-D= 0.919274); Simpson’s reciprocal index (1/D=12.38763726)

Table 3. Monthly based variations in the number of individuals, species richness, relative abundance (%) and biodiversity indices of the fishes found in Panjkora River of District Dir (Upper and Lower) of KPK province of Pakistan

| Months    | n  | SR | RA (%) | D     | H     |
|-----------|----|----|--------|-------|-------|
| March     | 60 | 6  | 8.28   | 0.19  | 1.65  |
| April     | 87 | 10 | 12.01  | 0.11  | 2.20  |
| May       | 255| 14 | 35.22  | 0.09  | 2.67  |
| June      | 142| 11 | 19.61  | 0.12  | 2.25  |
| July      | 110| 13 | 15.19  | 0.09  | 2.38  |
| August    | 52 | 6  | 7.18   | 0.22  | 1.58  |
| September | 18 | 2  | 2.48   | 0.49  | 0.66  |

Note: n = number of individuals; SR = species richness; RA = relative abundance; D = Simpson index of diversity; H = Shannon-Weiner index of diver.

**Biodiversity and microplastics pollution in the finfish species at KPK, Pakistan**

and also reported 20 species, which were belonging four families and three orders. Akhtar et al. (2014) also described 18 fish species belonging to the three families and three orders from rivers Swat of Khyber Pakhtunkhwa province of Pakistan. Hasan et al. (2015) had also reported about 22 fish species belongs to 10 families from lentic water bodies of Indus River at Ghazi Ghat of Pakistan. Ali et al. (2010) identified 15 fish species were belonging to the 13 genera and 5 families from Suleman Mountain Range of D.G. Khan. Hasan et al. (2013) had reported 16 fish species belongs to Four orders, 12 genera, and 5 families from Bajour Agency. Akhtar et al. (2014) had reported 10 fish species, and also a new record *Cyprinus carpio* from river Barandu in district Buner of KPK, Pakistan. Ali et al. (2010)
had also reported 15 fish species belongs to 13 genera and five families from freshwater bodies of Suleman Mountain Range of Dera Ghazi Khan Region of Pakistan.

In this study, a total of 120 fish individuals collected from the each species. Microplastics were found in 12 of the 120 examined fish. A total of 10 microplastic items were recovered from the 100 individuals: 4 microplastics from the gastrointestinal tract (25%), 6 items from the gills (50%) and 4 from the muscle (25%). Based on microplastic size, all the species had more fibers in the size range 100-400 mm in the gastrointestinal tract and 98-140 mm in gills. In the dorsal muscle, in the size range 80-600 mm (Lusher et al., 2013; Ory et al., 2018) the highest microplastics (90%) observed in site 7 and (10%) microplastics found in site 4 and 5. No microplastics were found in sites 1, 2 and 3. According to the recent study the site 7 is contaminated site because medical and general store discarded a lot of polythene bags in the river. The plastics have the chemical composition from which they are made (Jabeen et al., 2017; Hassan et al., 2023).

High incidences of environmental pollution and polymerization processes causes contamination reach the food chain, dangerous long-term carcinogenicity. Impact on fish, animals and humans due to the release of chlorofluorocarbons (cfcs), formaldehyde, lead, mercury and cadmium. The accumulation of polyethylene bags affected on survival and growth of fishes, like habitat conditions, development pattern, early life, feeding, fish fatness, degree of stomach completeness, gonad middle age, physical condition, overall health, gastrointestinal tract, dorsal muscle, lipid oxidative damage in the brain gills and increased brain AChE activity in fish containing microplastics similar finding (Hester-Ronald and Harrison, 2011; Hassan et al., 2023).

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