



Spatial variation in the otolith shape of Asian sheat catfish, *Wallago attu* (Bloch & Schneider, 1801) populations from five Indian rivers

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Abstract

The population of *Wallago attu* (Bloch & Schneider, 1801) has declined worldwide including in the rivers of India and has been declared 'Vulnerable' by the International Union for Conservation of Nature. The restoration and conservation of fishes is heavily dependent on their population status in their natural habitats. Therefore, otolith shape was used to study the stock structure of *W. attu* from rivers Ganga, Gomti and Yamuna in Northern India, river Hooghly in Eastern and Pampa in Southern part of India. Shape indices of the asteriscus otoliths and elliptical Fourier analysis of the contour shapes was investigated using univariate and multivariate statistical techniques. The otolith descriptors (otolith length, breadth, area and perimeter) and four shape indices (length-breath ratio, form factor, circularity and ellipticity) of the otoliths were different (ANOVA, $p < 0.05$) between the fish of five sampled rivers. Principal component analysis of normalized elliptical Fourier descriptors (NEFDs) of the contour shape accounted for 89.55% of the total variance. Discriminant function analysis on the basis of NEFDs resulted in 100% classification rate with no intermixing between the fish of different rivers and depicted the presence of different stocks of *W. attu* in a scatter plot of DF-I against DF-II. These variations in the otolith shape in the current study might be due to the restricted movement of fish because of geographical isolation and different environmental conditions in different rivers due to changes in habitat, or both. The current study would be helpful in developing effective strategies for stock management of this species.

Keywords: catfish; Fourier analysis; otolith; river; stock; *Wallago attu*

1 | INTRODUCTION

Otoliths (ear-stones or crystals) are the characteristic features of all teleost fishes located in the inner ear cavity and is considered to be an authentic phenotypic marker for the sequestration of species or populations because of the variations in its shape (Tuset *et al.* 2008; Reichenbacher *et al.* 2009; Tuset *et al.* 2015). It is a three-dimensional structure but not necessarily to grow at the same rate similarly in all dimensions and the shape and

size differ greatly among species (Campana and Thorrold 2001). Fish otoliths show mainly two processes, equilibrium and audition, which represent stable structures that are not subject to short-term variations caused by feeding conditions or reproduction (Cadrin *et al.* 2005). The study revealed that the ear of fish has three otolithic organs such as saccule, utricle and lagena, which play an important role in fishes to hear and to maintain the balance. Otolith is a type of "black box" that records the life histo-

ry features of an individual (Lecomte-Finiger 1999). The otolith shape is species specific and the identification of fish species based on otolith morphology (most often, on the structure of the largest otolith, the sagitta) is possible with the use of several regional atlases and keys (Campana 2004). Calcium carbonate (CaCO_3) is present in the structure of otoliths immersed in a non-collagenous organic matrix composed of acidic proteins and polysaccharides (Berra and Aday 2004; Popper *et al.* 2005). Otolith grows continuously throughout the life of a fish and remains metabolically static (Campana 1999). Otoliths play a significant role to record the chemical elements obtained by fishes from various water bodies during their life (Elsdon *et al.* 2008). Otolith form changes are caused by a wide range of diverse ecological parameters, including temperature, salinity, depth, substrate, and feeding conditions (Tuset *et al.* 2015). Otolith shape is potentially a strong beacon of distinct stocks. The use of otolith shape as a marker of fish population to differentiate the stocks has been investigated by many workers (Campana and Casselman 1993; Mériçot *et al.* 2007; Zhao *et al.* 2017; Moreira *et al.* 2019; Yang *et al.* 2024). The morphometric approaches utilized in otoliths are thought to be practical and affordable when compared to physiological and molecular techniques.

Wallago attu (Bloch & Schneider, 1801) is an economically important inland water teleostean fish belongs to family siluridae of the order siluriformes. It is commonly known as “Asian sheat catfish” and is fast growing species across the south and south-east Asian rivers (Montana *et al.* 2011; Renjithkumar *et al.* 2011). The fish is broadly distributed in India, Pakistan, Nepal, Afghanistan, Myanmar and Indonesia and also found across the rivers, reservoirs and watersheds of Thailand, Cambodia, Burma, Kampuchea, Vietnam, Malay Peninsula, Afghanistan, Sri Lanka, Sumatra and Java (Talwar and Jhingran 1991; Froese and Pauly 2021). Due to its high nutritional value, *W. attu* has good demand as a food fish in the market (Rufus *et al.* 2015). The enhanced customer demand of this fish followed in the progress of rigorous aquaculture of this species in Asian countries. It has been reported that the population of this fish is declined due to several factors like overfishing, predatory nature, catastrophic factor etc. (Patra *et al.* 2005; Montana *et al.* 2011) and has been enlisted as ‘Vulnerable’ in International Union for Conservation of Nature (IUCN) Red List of threatened species (Ng *et al.* 2019) and thus effective fishery management and restoration of this species in wild habitat is necessary in the current aspects.

Apart from the works of Kumar *et al.* (2023) that claims to be the first report on the body morphometrics of *W. attu* from different rivers in India using traditional truss analysis and modern geometric morphometrics, still there is no information on the otolith shape of this fish. Keeping in mind the current situation of this species in

India, the present work focused to study the otolith shape of *W. attu* collected from different rivers of India.

2 | METHODOLOGY

2.1 Sampling

Altogether 75 specimens of *W. attu* were collected from five different rivers in Northern (Ganga, Yamuna, Gomti), Eastern (Hooghly) and Southern (Pampa) India with the help of local fisherman between November 2021 and November 2022 (Table 1; Figure 1). The river Ganga emerges in the Garhwal Himalayas at an altitude of 4100 m above the sea level whereas the river Yamuna which is the second largest tributary of the Ganga and the longest tributary in India originates from the Yamunotri Glacier of Himalayas and meets Ganga at Sangam, Allahabad (Singh *et al.* 2022). River Gomti is also a tributary of Ganga originates from the Fulhar Jheel Gomat Taal, Pilibhit town in Uttar Pradesh and meets Ganga at Ghazipur (Uttar Pradesh). River Hooghly also known as ‘Kati-Ganga’ is an approximately 260 kilometre long distributary of the Ganga River in West Bengal and river Pampa originates at Pula-chimalai hill in the Peerumedu plateau in the Idukki district of Kerala (Kumar *et al.* 2023).

TABLE 1 Descriptive summary of the collected *Wallago attu* specimens.

Rivers	Sampling sites	Sample size	TL range, cm
Ganga	Kanpur	15	29.5–37.5
Gomti	Lucknow	15	20.5–45.2
Yamuna	Agra	15	19.4–33.6
Hooghly	Kolkata	15	20.3–38.4
Pampa	Kerala	15	37.2–51.2

TL = total length

2.2 Otolith removal and decontamination

Asteriscus otoliths (right and left) were extracted, collected in sterile plastic vial and brought to the laboratory. All equipments and glassware were cleaned with analytical grade 1% nitric acid (HNO_3) before decontamination. To eliminate the surface contamination, otoliths were immersed in 3% hydrogen peroxide (H_2O_2) for 5 min and dipped for 5 min in 1% HNO_3 . Otoliths were then deluged with Milli-Q water for 5 min to remove the acid. After decontamination, the otoliths were dried and stored in acid-washed vials for further image analysis (Khan *et al.* 2012).

2.3 Image acquisition

Otolith images were viewed under a stereo microscope (Leica MZ12) with sulcus up and images were captured using a digital camera attached to the microscope (Figure 2). The terminology of otolith morphology was described as given by Smale *et al.* (1995) and Tuset *et al.* (2008).

2.4 Otolith morphological indices

With the help of software ImageJ version 6.0 otolith length (OL, from anterior to posterior end), otolith breadth (OB, from dorsal to ventral side), perimeter (P) and otolith area (OA) were measured from each otolith image. Six shape indices Length-breadth ratio (OL/OB), Circularity (P^2/OA), Rectangularity ($OA/(OL*OB)$), Roundness ($4OA/(\pi OL^2)$), Form-factor ($4\pi OA/P^2$) and Ellipticity ($(OL-OB)/(OL+OB)$) were calculated (Tuset *et al.* 2003). The effect of size on otolith measurements was eliminated by using allometric formula as per Elliot *et al.* (1995):

$$M_{adj} = M * (L_s/L_o)^b$$

where, M_{adj} is new size-

adjusted parameter, M is the original measurement, L_s is the overall mean of the standard length (SL) for all fish from all the samples, L_o is the SL of the fish and parameter b is slope of the regression analysis of $\log_{10} M$ on $\log_{10} L_o$ for each parameter.

Differences in otolith descriptors between the left and right otoliths tested by students't-test showed no significant difference ($p > 0.05$), hence rest of the analysis was carried out using the right otolith. Univariate analysis of variance (ANOVA) was carried out to find the significant ($p < 0.05$) morphological shape indices between the fish of different rivers.

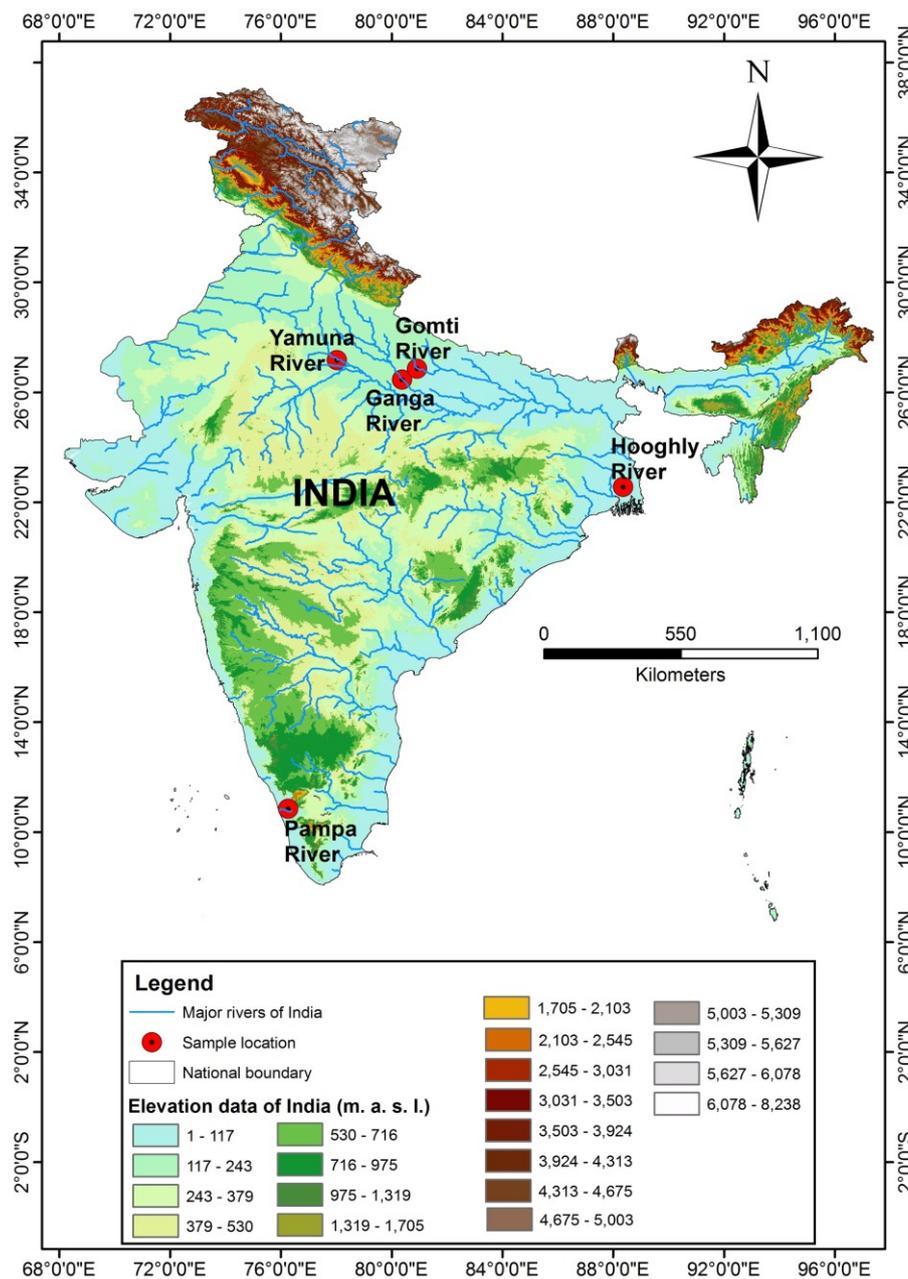


FIGURE 1 Map of the study area (Kumar *et al.* 2023) showing geographical locations of the sampling sites of *Wallago attu* from five rivers of India.



FIGURE 2 Asteriscus otolith of *Wallago attu*.

2.5 Elliptical Fourier analysis

The software package 'SHAPE' (Iwata and Ukai 2002) containing four programs (ChainCoder, Chc2Nef, PrinComp and PrinPrint) was used to study contour shapes of otolith based on elliptic Fourier descriptors (EFDs). EFDs (Kuhl and Giardana 1982) helps in delineation of biological shapes with 2D closed contours. Images were processed to high contrast and gray scale for contour extraction. Chain codes (Freeman 1974) was extracted by ChainCoder program for storing contour information of otoliths. Chain coded contours were transformed to normalized EFDs by the software Chc2Nef. Principal component analysis (PCA) of the coefficient of the EFDs was carried out to study shape variation in the data and visualization was achieved by PrinComp program. Finally, discriminant function analysis (DFA) was carried out for population discrimination.

3 | RESULTS

None of the morphological parameters of otolith were

significantly correlated to the SL of fish after allometric transformation. Thus, the effect of size on otolith was successfully removed from the otolith measured parameters. Details of the morphological parameters are given in Table 2. All measured parameters (otolith length, otolith width, perimeter and area) and four shape indices (length-breath ratio, form factor, circularity and ellipticity) were significantly different (ANOVA, $p < 0.05$) between the *W. attu* populations from five different rivers (Table 3).

Fourier analysis resulted in generation of 80 harmonics or elliptic Fourier descriptors (EFDs) for each otolith contour, out of which the four coefficients of each harmonics were normalized for size effect, rotation and start point. Thus, normalization of EFDs resulted in 77 normalized elliptic Fourier descriptors (NEFDs) for each otolith contour where the first three coefficient were normalized as $a_1=1$ and $b_1=c_1=0$. PCA of the 77 NEFDs for shape variation resulted in extraction of 10 effective principal components explaining 89.55% of total variance. Maximum variance was accounted by PC1 (41.63%) followed by PC2 (16.42%) and PC3 (9.62%). Reconstruction of otolith contour depicted by each principal component is given in Figure 3. Discriminant function analysis (DFA) of NEFDs between the populations yielded 4 discriminant functions (DF1, DF2, DF3 and DF4) of which 3 were highly significant ($p < 0.001$) accounting for 65.69%, 26.53% and 6.01% of total variance (Table 4). Group classification in DFA resulted in 100% classification of otolith specimens into their respective river population and clearly depicted five different populations in the scatter plot of DF1 against DF2 (Figure 4).

TABLE 2 Mean (\pm standard deviation) of descriptive parameters and shape indices of otoliths in *Wallago attu* from five rivers.

Parameters	Rivers				
	Gomti	Ganga	Yamuna	Hooghly	Pampa
Otolith length	4.03 \pm 0.68	3.58 \pm 0.28	4.02 \pm 0.42	4.65 \pm 0.53	4.84 \pm 0.26
Otolith breadth	2.19 \pm 0.30	1.89 \pm 0.15	2.17 \pm 0.17	2.34 \pm 0.22	2.33 \pm 0.19
Area	7.06 \pm 1.94	5.61 \pm 0.61	7.10 \pm 1.07	9.06 \pm 1.41	9.23 \pm 0.93
Perimeter	13.38 \pm 2.16	10.76 \pm 0.59	12.47 \pm 1.03	13.87 \pm 1.34	15.30 \pm 1.18
Length-breath ratio	1.84 \pm 0.12	1.90 \pm 0.12	1.85 \pm 0.15	1.99 \pm 0.14	2.08 \pm 0.15
Form-factor	0.49 \pm 0.06	0.61 \pm 0.04	0.57 \pm 0.03	0.59 \pm 0.02	0.50 \pm 0.04
Circularity	26.01 \pm 4.03	20.73 \pm 1.44	22.03 \pm 0.98	21.39 \pm 0.62	25.56 \pm 3.66
Roundness	0.55 \pm 0.04	0.56 \pm 0.10	0.56 \pm 0.05	0.53 \pm 0.06	0.50 \pm 0.04
Rectangularity	0.79 \pm 0.04	0.84 \pm 0.12	0.81 \pm 0.04	0.83 \pm 0.04	0.82 \pm 0.04
Ellipticity	0.29 \pm 0.03	0.31 \pm 0.03	0.30 \pm 0.04	0.33 \pm 0.03	0.35 \pm 0.03

TABLE 3 Significant descriptors and shape indices between the *Wallago attu* of five different rivers of India.

Parameters		Sum of square	df	Mean square	F	p-values
Area	Between Groups	139.286	4	34.822	21.405	<0.001
	Within Groups	113.878	70	1.627		
	Total	253.164	74			

TABLE 3 Continued.

Parameters		Sum of square	df	Mean square	F	p-values
Perimeter	Between Groups	170.443	4	42.611	23.043	<0.001
	Within Groups	129.444	70	1.849		
	Total	299.888	74			
Length	Between Groups	15.727	4	3.932	18.596	<0.001
	Within Groups	14.800	70	0.211		
	Total	30.527	74			
	Between Groups	2.010	4	0.503	11.316	<0.001
	Within Groups	3.109	70	0.044		
	Total	5.119	74			
L-B ratio	Between Groups	.629	4	0.157	8.194	<0.001
	Within Groups	1.344	70	0.019		
	Total	1.974	74			
Form factor	Between Groups	0.168	4	0.042	19.828	<0.001
	Within Groups	0.148	70	0.002		
	Total	0.315	74			
Circularity	Between Groups	362.405	4	90.601	13.696	<0.001
	Within Groups	463.055	70	6.615		
	Total	825.460	74			
Roundness	Between Groups	0.036	4	0.009	2.380	0.060
	Within Groups	0.268	70	0.004		
	Total	0.305	74			
Rectangularity	Between Groups	0.022	4	0.006	1.371	0.253
	Within Groups	0.281	70	0.004		
	Total	0.303	74			
Ellipticity	Between Groups	0.033	4	0.008	7.780	<0.001
	Within Groups	0.074	70	0.001		
	Total	0.107	74			

Significant values are shown in bold

TABLE 4 Discriminant functions and results of Wilks' lambda test verifying shape variation on the basis of NEFDs of otoliths.

Functions	Eigen value	Variance (%)	Wilks' lambda	Chi-square	df	p-value
DF1	302.29	65.69	0.000	587.449	280	<0.001
DF2	122.10	26.53	0.000	378.863	207	<0.001
DF3	27.65	6.01	0.004	203.188	136	<0.001
DF4	8.13	1.77	0.11	80.725	67	0.121

Significant values are shown in bold

4 | DISCUSSION

Otolith shape study has been extensively used for stock discrimination and population structuring (Tuset *et al.* 2003; Turan *et al.* 2006; Jemaa *et al.* 2015; Vasconcelos *et al.* 2018). The elliptical Fourier analysis to study otolith contour for shape variation is a strong technique for overall shape and minute differences in the otolith shape (Campana and Casselman 1993; Vieira *et al.* 2014). NEFDs are size-free variables that are normalized for otolith shape, rotation and starting point (Iwata and Ukai 2002).

In the present study, there was no significant difference between the left and right otolith of the fish. Many workers also reported the same in different fish species (Megalofonou 2006; Valinassab *et al.* 2012; Félix *et al.* 2013; Zengin *et al.* 2015; Singh *et al.* 2022). Significant variation in the otolith morphology on the basis of otolith

parameters (OL, OB, A and P) and shape indices (Length-breadth ratio, Circularity, Rectangularity, Roundness, Form-factor and Ellipticity) between the different river groups indicated variation in the otolith shape. Further, elliptical Fourier analysis based on NEFDs of otolith contour shape discriminated the *W. attu* of the five rivers in DFA with 100% group classification rate and scatter plot of DF1 against DF2 in DFA clearly indicated the presence of five different phenotypic populations of *W. attu*.

Geographical isolation, biological barriers, differences in the environmental conditions and/or genetic factors might be responsible for the current variations. Large geographical distance between the sites promotes population differentiation and biological breaks results in variation of the phenotypic characters in the populations (Barria *et al.* 2014; Vieira *et al.* 2014).

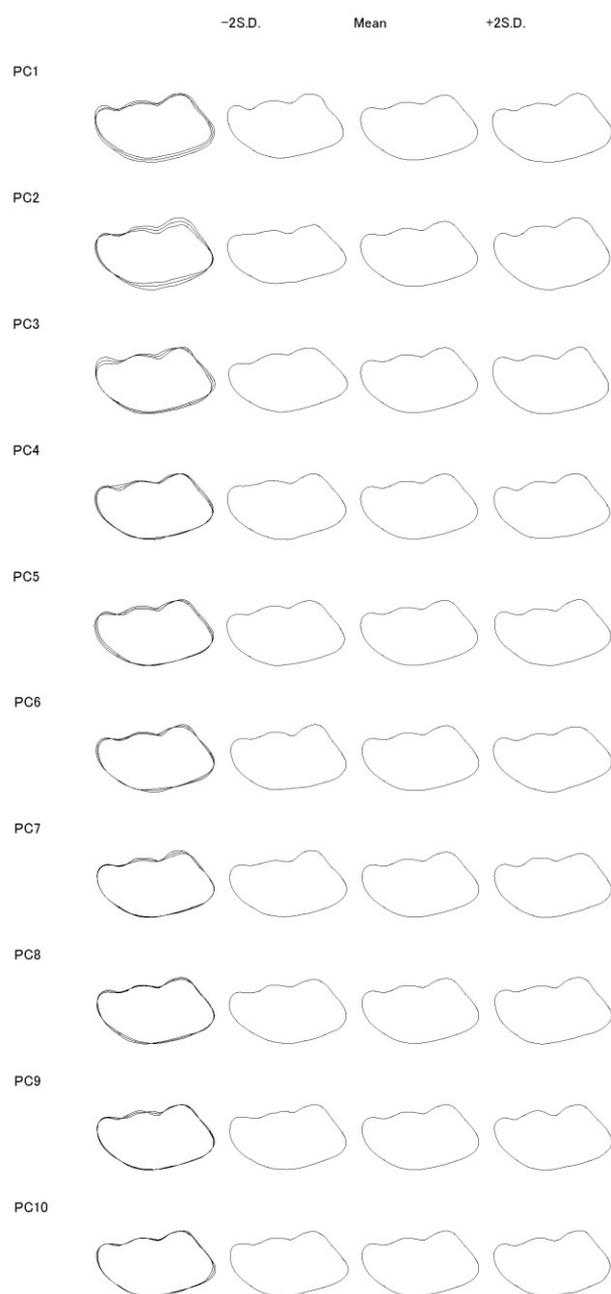


FIGURE 3 Contour variations in otolith of *Wallago attu* in principal component analysis of the normalized elliptical Fourier descriptors (NEFDs).

In the current study the sampling locations of five different rivers Ganga, Gomti, Yamuna, Hooghly and Pampa at Kanpur, Lucknow, Agra, Kolkata and Kerala are geographically isolated distance sites. Kanpur, Lucknow and Agra are three different districts in Northern part of India, where as Kolkata and Kerala lie in the eastern and southern part of India respectively. The distribution of the river systems affects the population structure of freshwater organisms (Ikeda *et al.* 1993; Hara *et al.* 1998). Rivers

Ganga, Gomti, Yamuna and Hooghly are integral part of Gangetic basin in Northern India and Pampa river basin in Southern India. Inter-basin and intra-basin variations in the current study are thus due to geographical isolation and river fragmentation respectively that isolates the fish of one river from another due to restricted movement.

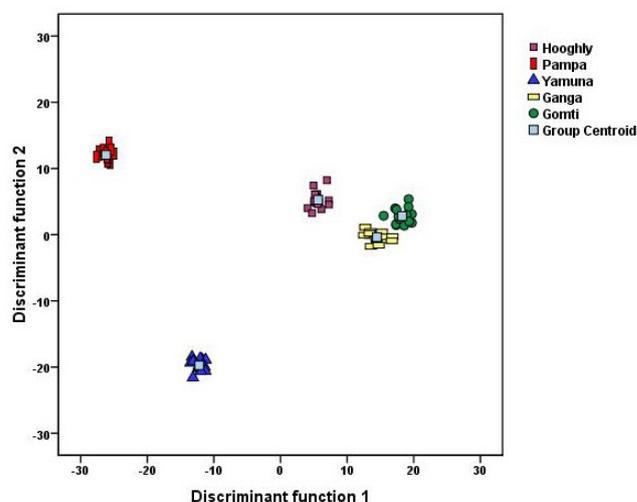


FIGURE 4 Discriminant function scatter plot on the basis of normalized elliptical Fourier descriptors (NEFDs) of otoliths in *Wallago attu* of five Indian rivers.

High genetic variation also results in the diversity within the population of a species inhabiting different environmental conditions for survival (Kumar *et al.* 2024). Phenotypic variations on the basis of truss and geometric morphometrics also discriminated the stocks of *W. attu* from five different rivers, but contrary to the present findings on otolith shape it showed eminent levels of homogeneity between the Ganga, Gomti and Hooghly samples (Kumar *et al.* 2023). Similar trends of homogeneity and heterogeneity in stock discrimination using body morphometrics and otolith shape respectively has been reported in *Channa punctatus* of river Ganga (Khan *et al.* 2013; Miyan *et al.* 2014). Thus, when compared to body morphometrics otolith shape gives a better stock delineation of *W. attu* of the rivers Ganga, Gomti and Hooghly.

5 | CONCLUSIONS

Spatial variations in the otolith shape of *W. attu* populations indicated the existence of five different stocks of *W. attu* that might be due the geographical isolation, differences in the environmental conditions and/or genetic basis. However, molecular investigations of *W. attu* populations from these rivers is required to explore the genetic basis of this stock discrimination. Use of genetic markers to examine basis of discreteness between the geographically different stocks would be useful for building effective management strategies for conservation of this vulnerable species.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTION

GK: Research design, sample collection and data, manuscript writing. AK: Data analysis and helped in preparation of first draft of manuscript. MS: Supervision, critical review and editing of the manuscript.

DATA AVAILABILITY STATEMENT

All the datasets generated and analyzed in this study are included in the manuscript and presented as tables and figures.

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