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Studies on the Elemental Profile of Otoliths and Truss Network Analysis for Stock Discrimination of the Threatened Stinging Catfish *Heteropneustes fossilis* (Bloch 1794) from the Ganga River and Its Tributaries

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Mohammad Afzal Khan, Kaish Miyan, Shahista Khan, Devendra Kumar Patel and Nasreen Ghazi Ansari (2012) Studies on the elemental profile of otoliths and truss network analysis for stock discrimination of the threatened stinging catfish *Heteropneustes fossilis* (Bloch 1794) from the Ganga River and its tributaries. *Zoological Studies* **51**(7): 1195-1206. The present study was undertaken to identify different stocks of the stinging catfish *Heteropneustes fossilis* inhabiting the Ganga, Yamuna and Gomti Rivers of India using the elemental profile of sagittal otoliths and truss network analysis of the entire body shape. Inductively coupled plasma-atomic emission spectrometry was used to estimate concentrations of 12 trace elements in otoliths, and 11 morphometric landmarks were chosen to construct a truss network of the fish in order to discriminate among stocks. A discriminant function (DF) analysis of elemental profiles showed that Ba, Pb, Zn, and Sr successfully discriminated *H. fossilis* populations from different rivers. DF-I, DF-II, and DF-III accounted for 76.1%, 17.9%, and 6%, respectively, of the among-group variability in the elemental profile of otoliths. In the analysis of truss landmarks, the principal components, PC-I and PC-II, respectively accounted for 39.1% and 13.4%, while DF-I and DF-II, respectively accounted for 59.7% and 25.5% of the among-group variability. The overall allocation success of individuals to their group of origin was high (98.7%) in the elemental profile of otoliths compared to truss measurements (72.3%). The truss network analysis distinguished separate stocks of fish in the 3 rivers; however, the elemental profile of otoliths further discriminated the stocks at the 2 sampling stations within the Ganga River. http://zoolstud.sinica.edu.tw/Journals/51.7/1195.pdf

Key words: Stock structure, *Heteropneustes fossilis*, Truss morphometry, Otolith chemistry, Indian rivers.

A fish stock may be defined as an intra-specific group of randomly mating individuals with temporal or spatial integrity (Ihseen et al. 1981). Information on the stock structure is useful for developing management strategies that will be helpful in conserving biodiversity of species, subspecies, stocks, and races (Turan et al. 2005). Stock identification is important for effective fisheries management (Kutkuhn 1981), because separate parts of a population can be separately managed for optimal yields. Poorly informed fishery management can lead to dramatic alterations in the biological attributes and productivity of a species (Altukhov 1981, Turan 2006).

We used elemental profiles of otoliths and fish morphometrics to delineate the probable stocks of the stinging catfish *Heteropneustes fossilis.* Environmental markers, such as the elemental composition of otoliths, have successfully been

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used to delineate stock structures (Thresher 1999, Campana et al. 2000). Differences in elemental compositions are usually thought to reflect environmental differences between groups of fish, although it was suggested that genetic effects may also contribute to differences among individuals, stocks, and species (Kalish 1989, Thresher et al. 1994). Otoliths are used as natural markers mainly because they are metabolically inert. Also, elemental compositions of otoliths are regulated by the physico-chemical environment in which the fish lives. Application of trace elemental compositions of otoliths as a natural tag is effective in identifying various stocks of fish species such as westslope cutthroat trout *Oncorhyncus clarki lewisi* (Wells et al. 2003), yellow perch *Perca flavescens* (Brazner et al. 2004), lake trout *Salvelinus namaycush* (Munro et al. 2005), Centrarchids; *Micropterus salmoides*, *Lepomis cyanellus*, *L. macrochirus* and *Pomoxis nigromaculatus* (Whitledge et al. 2007), and *Micropterus salmoides*, *M. punctulatus*, *Lepomis cyanellus*, *L. macrochirus*, *L. humilis*, *Pomoxis nigromaculatus*, *Morone mississippiensis*, *M. chrysops*, and *Aplodinotus grunniens* (Zeigler and whitledge 2010), striped bass *Morone saxatilis* (Schaffler and Winkelman 2008). Studies on phenotypic variations among populations continue to play an important role in stock identification, in spite of the advent of biochemical and molecular genetic techniques which have identified neutral genetic differences between groups (Swain and Foote 1999, Turan 2004). Fish morphometry has been used to identify stocks of many fishery resources (Cadrin and Silva 2005, Saini et al. 2008, Sajina et al. 2011).

The stinging catfish *Heteropneustes fossilis* (Bloch 1794) is a valuable food fish with high market demand across India on account of its taste and nutritional value. The culturing of this fish has not yet been taken up on a commercial scale in India, and almost all consumer demand is met through wild catches. The wild population of this fish species is rapidly declining due to overexploitation, the introduction of alien species, loss of habitat, diseases, water pollution, water abstraction, siltation, poisoning, etc. and the fish has consequently been listed as vulnerable (Molur and Walker 1998). The fish is distributed in rivers, lakes, ponds, and derelict water bodies of India, Sri Lanka, Pakistan, Nepal, Bangladesh, Burma, Thailand, and Laos (FishBase 2011).

There is a paucity of information on the stock structure of *H. fossilis* from Indian rivers, and practically no information on the use of otolith chemistry and truss network analysis to identify stocks of this fish species. Moreover, there are no published reports available on the use of the elemental profile of otoliths to discriminate stocks of any freshwater or marine fish from India. Therefore, the present study was undertaken to discriminate probable stocks of *H. fossilis* inhabiting 3 Indian rivers, based on elemental profiles of otoliths and fish morphometric analysis.

MATERIALS AND METHODS

Sampling

In total, 204 *H. fossilis* specimens were investigated from the Ganga, Yamuna, and Gomti Rivers (Fig. 1). Samples were collected from 40 fish from Narora (27°-30'N, 78°-25'E) and 50 fish from Kanpur (26°-28'N, 80°-24'E) on the Ganga river, 48 fish from Firozabad (27°-09'N, 78°-24'E) on the Yamuna River and 66 fish from Lucknow (26°-55'N, -80°-59'E) on the Gomti River. Morphological identification of the fish was based on a description by Talwar and Jhingaran (1991).

The Ganga River originates in the Garhwal Himalayas (30°-55'N, 70°-7'E) at an elevation of 4100 m from the Gaumukh glacier in Uttrakhand, India. It flows about 2525 km before reaching the sea (Kamyotra 2009). The Yamuna River, which originates from the Yamunotri glacier (Saptrishi Kund) near Bander Punch peaks (38°-59'N, 78°-27'E) at an elevation of about 6320 m in the Mussoorie range of the lower Himalayas in Uttarkashi district of Uttarakhand, India, traverses about 1336 km to merge with the Ganga River at Allahabad in Uttar Pradesh State, India, (Sengupta 2006). The Gomti River, originating from a natural lake (28°-34'-N, 80°-07'-E) near the town of Pilibhit in Uttar Pradesh about 50 km south of the Himalayan foothills, travels about 750 km before merging with the Ganga River near Varanasi in Uttar Pradesh, India (Sarkar et al. 2010). Among the 3 selected rivers, the Yamuna and Gomti Rivers are the tributaries of the Ganga River.

Morphometric measurements

Only undamaged specimens were used for morphometric measurements. A truss network was constructed as the network on the fish body as described by Strauss and Bookstein (1982). The truss analysis quantifies the multidimensional shape of a fish. With this technique, the body

shape of a fish is described by distances between pairs of landmarks across the body, which form a sequential series of connected polygons called a truss box. The truss network can systematically be used to detect shape differences in oblique, horizontal, and vertical directions (Strauss and Bookstein 1982). A fish was placed on a water-resistant sheet, and measurements were taken by piercing the paper with a needle at corresponding anatomical landmarks. Eleven morphometric landmarks were chosen which determined the 23 interlandmark distances as shown in figure 2. Measurements were taken immediately after the fish samples were collected. Additional data such as the total length (TL), standard length (SL), head length (HL), post orbital length (POL), eye diameter (ED), body depth (BD), pectoral fin length (PFL), and ventral fin length (VFL) were also recorded.

Otoliths removal and sample preparation

Sagittal otoliths were removed from the otic capsules by opening the otic bulla after the morphometric measurements had been taken. The otoliths were placed in plastic vials, taken to the laboratory, and stored in paper envelopes. For the elemental profile of otoliths, 20 otoliths each were taken from fish collected from Narora and Kanpur on the Ganga River, and from Lucknow on the Gomti River. However, only 15 otoliths were taken for analysis from fish collected at Firozabad on the Yamuna River.

Otoliths (sagittae) were cleaned of surface contaminants. All implements and glassware were cleaned with analytical grade 1% nitric acid (HNO_3) before decontamination. Otoliths were soaked in ultra-pure water to remove all adhering biological

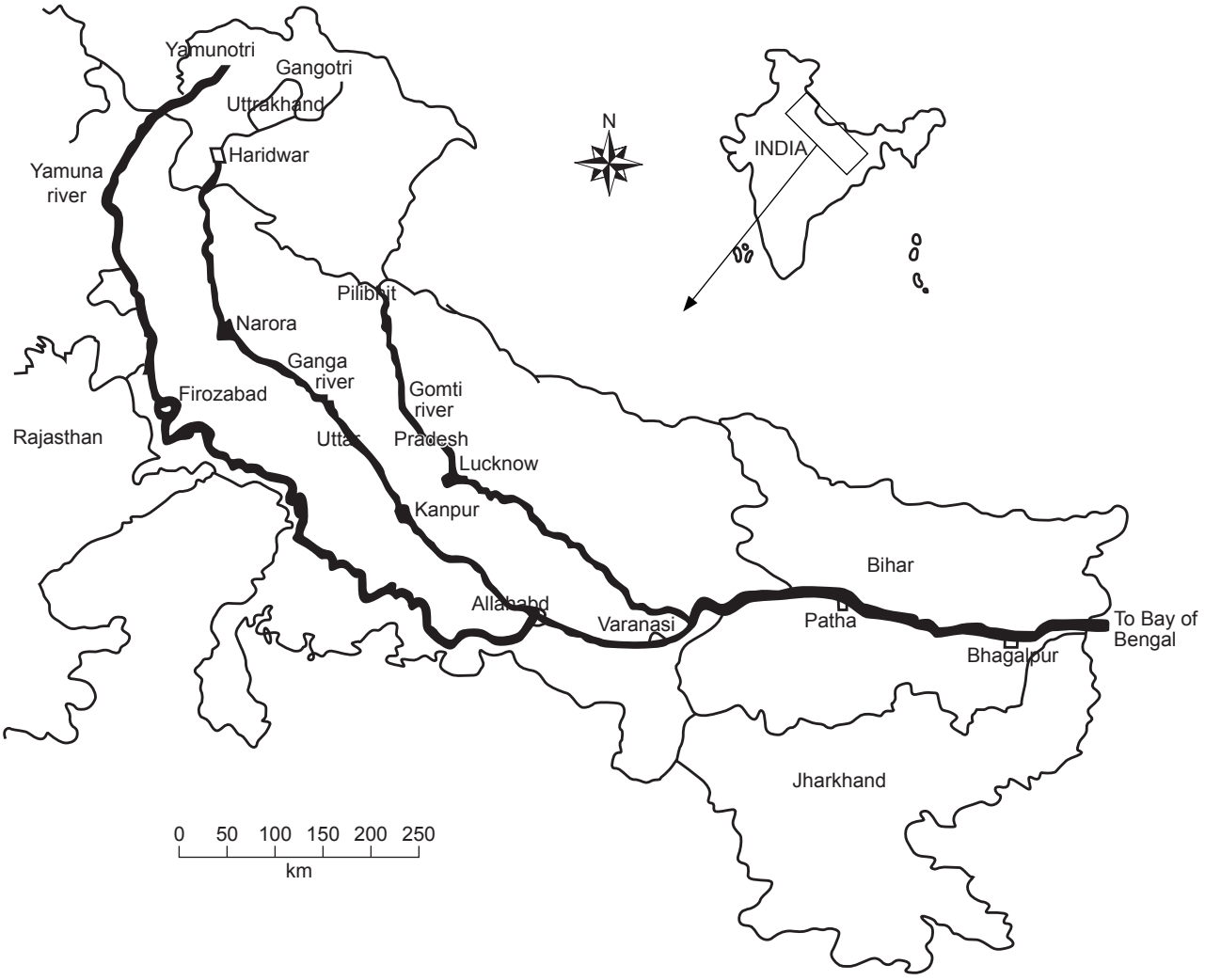

Fig. 1. Map showing collection sites of *H. fossilis* from the Ganga River and its tributaries: the Yamuna and Gomti Rivers.

residues. To dissolve the remaining biological residues, otoliths were soaked in 3% hydrogen peroxide for 5 min and immersed for 5 min in 1% HNO_3 to remove surface contaminants. To remove the acid, otoliths were then flooded with ultra-pure water for 5 min. Finally, otoliths were dried under a laminar flow hood and weighed to the nearest 0.1 mg. The decontaminated otoliths were stored dry in sealed, acid-washed polypropylene vials until analysis (Turan 2006).

Elemental analysis

Decontaminated otoliths were dissolved in 10 ml of 37% HNO_3 and the volume was brought up to 25 ml with deionized water. The elements (Ca, Na, Mg, Sr, Ba, Pb, Mn, Fe, Li, K, Cd, Cr, Cu, Ni, and Zn) were analyzed using inductively coupled plasma-atomic emission spectrometry (ICP-AES) (IRIS, Intrepid II XDL duo, Thermo Electronic, Waltham, MA, USA). Different elements were determined using different spectral lines. Standards were prepared with high-purity multi-element standard IV, obtained from Accu Standards (New Haven, CT, USA; traceable to NIST reference standards) using ultra-pure Milli Q water (Millipore Corporation, Billerica, MA, USA) and 2% v/v analytical grade HNO_3. A calibration blank was also prepared in a similar manner. Five points were obtained to create a calibration curve. Concentrations of elements in the sample and blank were calculated and are expressed as μg/g on a dry-weight basis.

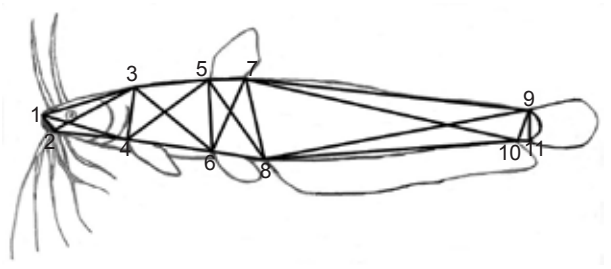

Fig. 2. Locations of the 11 landmarks for constructing the truss network system of *H. fossilis*. Landmarks refer to (1) anterior tip of the snout at the upper jaw, (2) posterior-most point of the maxillary, (3) posterior-most aspect of the neurocranium, (4) origin of the pectoral fin, (5) origin of the dorsal fin, (6) origin of the ventral fin, (7) insertion of the dorsal fin, (8) origin of the anal fin, (9) anterior attachment of the dorsal membrane from the caudal fin, (10) insertion of the anal fin, and (11) anterior attachment of the ventral membrane from the caudal fin.

Data analysis

Elemental profile of otoliths

The raw data were checked for outliers that would indicate the possible presence of broken otoliths or measurement errors. However, no outliers were recorded. Correlations were carried out between elemental concentrations and the SL of fish to check the effects of the SL on elemental concentrations. Significant correlations were observed between the fish size and elemental concentrations of samples. On account of the size effect, the data were standardized according to Bergenius et al. (2005) to remove the effect of the SL from each elemental concentration:

$$C_{ij\ adj} = C_{ij} \pm b\ (L_{S\ ij} - L_{S\ Mi})$$

where $C_{ij\ adj}$ is the sample concentration of fish i adjusted to the mean SL of group j, C_{ij} is the sample concentration of fish i from group j, b is the slope of the relationship of C_{ij} to $L_{S\ ij}$ common to all groups, $L_{S\ ij}$ is the SL of fish i in group j, and $L_{S\ Mi}$ is the average SL in group j. The adjusted concentrations for all elements of interest were then analyzed by a multivariate analysis of variance (MANOVA) to test for spatial differences in the multivariate elemental signatures. The assumption of homogeneity of variance of each dependent variable was examined using Levene's test, and the homogeneity of the group covariance matrix was examined using Box's M test (MANOVA). Data were tested for normality using the Kolmogorov-Smirnov test. A univariate analysis of variance (ANOVA) was used to explore patterns of each of the elements separately when significant effects were indicated in the MANOVA. Wilk's lambda criterion was used to test for group differences in the MANOVAs. A stepwise discriminant function (DF) analysis (DFA) was used to examine the elemental signatures in discriminating populations among the sites and to investigate whether signatures could be used to classify samples into their original group. A leave-one-out classification was carried out to assign individuals to their original group. Scatterplots of the 1st 2 discriminant scores were drawn to visualize the separation on the graph.

Truss morphometry

As most measures of shape are in some way related to size, any heterogeneity in the size

across samples will result in heterogeneity in shape without providing information on differences in body proportions among populations (Reist 1985). Since the SL of fish specimens varied, it was necessary to remove differences due to size variations. All morphometric measurements were standardized according to Elliot et al. (1995) to eliminate the size component from the shape measurements:

$$M_{adj} = M(L_s/L_o)_b$$

where, M is the original measurement, M_{adj} is the size-adjusted measurement, L_o is the SL of the fish, and L_s is the overall mean SL for all fish from all samples in each analysis. Parameter b was estimated for each character from the observed data as the slope of the regression of log M on log L_o. The transformed data were checked by testing the significance of the correlation between the transformed variables and the SL. The SL was excluded from the final analysis.

Transformed data were analyzed using a principal component (PC) analysis (PCA) and DFA to examine any phenotypic differences between populations. The PCA was used to examine which of the morphometric measurements could differentiate populations. Eigenvectors and eigenvalues were obtained from the covariance matrix from the PCA which allowed for the largest part of the variance of the original variables with a low number of factors. This analysis enabled the evaluation of the relation between populations by means of the proximity in the space defined by components. The DFA was used to assign individuals to their original group. A stepwise procedure was used to reduce the number of variables in order to meet the requirement of a reduced set of characters for the DFA. The ability of the phenotypes to discriminate between populations was assessed by a cross-validation test. The basis for differentiating among samples was based on the percentage of correctly and incorrectly classified fish. The measure of the morphological distinctness of a population was given by the percentage of correctly classified individuals. The degree of intermingling between the populations was shown by the number of misclassified individuals. A univariate ANOVA was performed to test whether there was any statistically significant difference in each morphometric character between populations. All statistical analyses were carried out with MS-Excel (Microsoft Corporation, Redmond, WA, USA) and SPSS vers. 16 (SPSS, Chicago, IL, USA).

RESULTS

Elemental profile of otoliths

Concentrations of the trace elements, Cd and Cr, were below detection limits in samples from all sites. Li was detected only in samples from the Gomti River (mean ± SE, 8.13 ± 1.15) and at Kanpur (mean ± SE, 3.1 ± 1.2) on the Ganga River. Elemental concentrations differed among the 4 sites (MANOVA, $p < 0.01$). Eight of 12 elements analyzed exhibited significantly (ANOVA, $p < 0.001$) different mean values (Table 1). Otoliths from Kanpur on the Ganga River showed significantly higher concentrations of Ca, Sr, Mn, Cu, and Zn compared to otoliths from the other sites (ANOVA, $p < 0.001$). A significantly higher ($p < 0.001$) concentration of Ba was found in otoliths collected from Narora on the Ganga River, while Pb was found to be significantly higher ($p < 0.001$) in otoliths from the Yamuna River. Ni was present in significantly higher ($p < 0.001$) concentrations in otoliths of fish collected from the Gomti and Yamuna Rivers.

The DFA of the data produced 3 DFs. The 1st DF (DF-I) accounted for 76.1% of the total variation. The 2nd (DF-II) and 3rd DFs (DF-III) respectively accounted for 17.9% and 6%, of the group variability among populations. In the DFA, elements Ba, Pb, Zn, and Sr contributed most to discriminating among the different populations. Allocation success was high for all populations with 98.7% of individuals being correctly classified into their original group using the DFA. Data obtained at Kanpur on the Ganga River, at Firozabad on the Yamuna River, and at Lucknow on the Gomti River exhibited 100% classification success. However, a degree of misclassification occurred with fish from Narora on the Ganga River (Table 2). A graphical representation of the 1st 2 DFs revealed a clear separation of the Narora, Kanpur, Firozabad and Lucknow populations (Fig. 3).

Truss morphometry

None of the standardized morphometric measurements showed a significant correlation with the SL, which indicated that the size effect had been removed from the data by the allometric transformation. The univariate ANOVA showed highly significant ($p < 0.001$) differences

between the means of 4 samples for 25 of 29 morphometric measurements. Morphometric measurements of 2-3, 2-4, POL, and PFL did not differ ($p > 0.01$) (Table 3). Standardized morphometric measurements explained 62.7% of the total variation in the PCA. The 1st PC (PC-I) accounted for 39.1% of the total variance, and it was positively correlated to some variables while negatively correlated to others, showing variation due to the body shape. The 2nd PC (PC-II) accounted for 13.4% and the 3rd PC (PC-III) for 10.1% of the total variation. The contribution to the 1st PC was from the morphometric measurements ED, HL, 1-3, 1-2, 5-6, 4-5, BD, 3-6, and 5-8 (Table 4).

The DFA revealed that the 1st DF (DF-I) accounted for 59.7% of the total variation, while DF-II and DF-III respectively accounted for 25.5% and 14.8%, of the group variability among populations. The scatter plot of DF-I vs. DF-II explained 85.2% of the total variance among samples and formed 3 different groups. Samples from the Narora and Kanpur populations on the Ganga River formed 1 group, while the 2 other groups were separately comprised of fish from the Yamuna and Gomti Rivers (Fig. 4). This indicated the existence of different stocks in these rivers. The overall allotment of individuals to their original population was high (72.3%) (Table 5). The percentage of correctly classified fish was found to be highest in the Yamuna River (83.3%) followed by those in the Gomti River (81.8%). The higher misclassification observed for the Narora (25%) and Kanpur (30%) sampling stations on the Ganga River may have been due to their spatially being very close to each other.

Table 1. Mean and standard deviation (S.D.) values, and results of an ANOVA of elemental concentration (μg/g) of otoliths of *H. fossilis* from the Ganga River and its tributaries: the Yamuna and Gomti Rivers

Elements	River Ganga (Narora)		River Ganga (Kanpur)		River Yamuna (Firozabad)		River Gomti (Lucknow)		Wilks lambda	F	*P* value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Ca	64.52	41.81	111.62	36.37	85.33	9.15	71.97	28.66	0.745	8.11	0.000*
Na	3.45	0.91	3.62	1.52	2.79	0.68	2.87	0.97	0.899	2.67	0.054
Mg	8.21	8.15	6.91	3.98	7.01	1.31	7.65	8.01	0.993	0.18	0.912
Sr	0.14	0.08	0.36	0.18	0.35	0.07	0.33	0.17	0.685	10.89	0.000*
Mn	0.07	0.06	0.14	0.08	0.09	0.02	0.08	0.04	0.802	5.85	0.001*
Ba	0.06	0.02	0.02	0.02	0.03	0.06	0.04	0.02	0.069	320.74	0.000*
Pb	0.37	0.14	0.67	0.15	0.78	0.16	0.25	0.11	0.295	56.61	0.000*
Fe	1.29	1.14	1.39	0.73	0.87	0.15	0.71	0.67	0.874	3.40	0.022
K	1.17	1.16	1.11	0.41	0.76	0.12	0.59	0.59	0.887	3.00	0.036
Cu	0.18	0.15	0.36	0.18	0.25	0.07	0.18	0.09	0.742	8.21	0.000*
Ni	0.03	0.01	0.04	0.01	0.05	0.01	0.05	0.01	0.780	6.69	0.000*
Zn	0.41	0.35	0.96	0.41	0.54	0.07	0.32	0.21	0.578	17.25	0.000*

*$P < 0.001$.

Table 2. Results of a discriminant function analysis (DFA) showing the number and percentage of individuals classified into each group for the elemental profiles of otoliths from the original matrix

	River Ganga (Narora)	River Ganga (Kanpur)	River Yamuna (Firozabad)	River Gomti (Lucknow)	Total
River Ganga (Narora)	19	1	0	0	20
%	95	5	0	0	100
River Ganga (Kanpur)	0	20	0	0	20
%	0	100	0	0	100
River Yamuna (Firozabad)	0	0	15	0	15
%	0	0	100	0	100
River Gomti (Lucknow)	0	0	0	20	20
%	0	0	0	100	100

DISCUSSION

Profiles of elemental concentrations across otoliths combined with the truss morphometric analysis indicated discrete populations of *H. fossilis* in the Ganga River and its tributaries: the Yamuna and Gomti Rivers. Otoliths were further discriminated among populations at the 2 sampling sites on the Ganga river. Higher concentrations of Ca, Na, Sr, Mn, Fe, Cu, and Zn in otoliths of *H. fossilis* in the Kanpur population might have been due to a higher availability of these elements in the Ganga River, because it is known that the river at Kanpur is grossly polluted by industrial effluents (Kamyotra 2009). About 80%-90% of Ca and Sr were derived from the surrounding waters in the case of freshwater fishes (Farrell and Campana 1996, Campana 1999). Ferguson et al. (2011) reported higher ratio of Sr:Ca in otoliths of *Argyrosomus japonicus* from an eastern region of the South Australia population and suggested that this may have been related to higher ambient Sr concentrations.

The population of *H. fossilis* at Narora on the Ganga River showed lower concentrations of elements in otoliths which might have been due to the better water quality relative to downstream stretches of the river. The major sources of elements at Narora are likely to be anthropogenic activities and surface run-off from agriculture fields containing fertilizers and pesticides. The higher concentration of Ba and lower concentration of Sr at Narora may have been due to the better water quality compared to other sites, as Ba indicates a terrestrial input to the water body. Campana (1999) and Elsdon et al. (2008) reported that Ba is a nutrient-type element and an indicator of availability of fresh water, while Sr is related to higher salinities. Longmore et al. (2010) reported that the highest concentration of Ba was detected in otoliths of *Coryphaenoides rupestris* from the Mid-Atlantic ridge of the North Atlantic ocean population which may be attributed to the availability of ambient Ba. Ferguson et al. (2011) reported a higher ratio of Ba:Ca in otoliths of *Argyrosomus japonicus* from an eastern region population in South Australia. A large number of glass industries pouring effluents into the Yamuna River at Firozabad may have contributed to the higher concentration of Pb in fish otoliths. An earlier study reported the heavy metals of Cd, Cr, Ni, Pb, Fe, Cu, and Zn in the Yamuna River (Sengupta 2006).

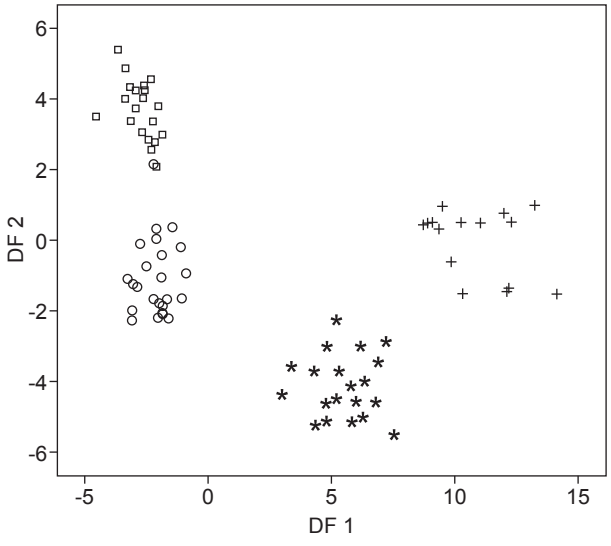

Fig. 3. Scatterplot of the 1st 2 canonical discriminant scores from the discriminant function analysis (DFA) for elemental profiles of otoliths of *Heteropneustes fossilis* collected from the Ganga River and its tributaries: the Yamuna and Gomti Rivers. ○, Ganga River (Narora); ▫ , Ganga River (Kanpur); +, Yamuna River (Firozabad); and ⋆, Gomti River (Lucknow).

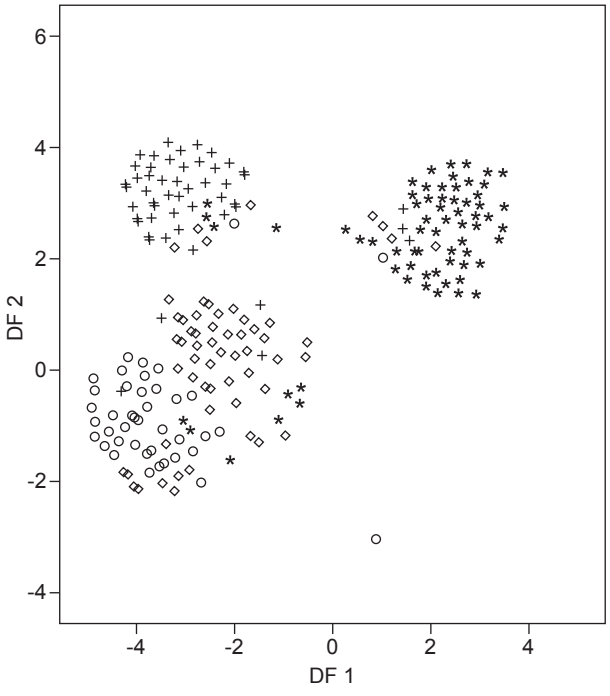

Fig. 4. Scatterplot of the 1st 2 canonical discriminant scores from the discriminant function analysis (DFA) for morphometric characters of *Heteropneustes fossilis* collected from the Ganga River and its tributaries: the Yamuna and Gomti Rivers. ○, Ganga River (Narora); ◇, Ganga River (Kanpur); +, Yamuna River (Firozabad); and ⋆, Gomti River (Lucknow).

The *H. fossilis* population from the Ganga River showed comparatively larger sizes of the head, mouth, eye diameter, and body depth which might be associated with the availability of food in the region, as argued by Rao (2001). Similar observations of larger sizes of the head, mouth, eye diameter, and pectoral fin length were also observed in a *Channa punctatus* population from the Ganga River, while the Yamuna River population exhibited a smaller body depth, mouth size, and pectoral fin length (Khan et al. 2012). The truss network analysis of *Megalaspis cordyla* along the Indian coast showed the possibility of 2 stocks with differences mainly noted from the anterior 1/2 and caudal area of the fish body (Sajina et al. 2011). Saini et al. (2008) reported a higher maximum body width, longer adipose, anal, and caudal fin, and an elongated caudal peduncle for the *Mystus seenghala* population in the Sutlej River, while the Beas River population of this fish showed a greater head depth, dorsal fin length and eye diameter. Stocks of *Labeo calbasu* from 2 isolated rivers and a hatchery were differentiated using truss morphometry and meristic characters by Hossain et al. (2010).

The Yamuna River population of *H. fossilis* showed a smaller body depth, mouth size, and measurements from the anterior portion of the body, which could possibly be attributed to the decreasing availability of food and a heavy pollution load. Cadrin and Silva (2005) reported that southern New England populations of yellowtail flounder, *Limanda ferruginea* had a shorter snout length, head length, and pectoral fin, compared to

Table 3. Mean and standard deviation (S.D.) values and results of an ANOVA for 29 morphometric characters of *H. fossilis* from the Ganga River and its tributaries: the Yamuna and Gomti Rivers

Characters	River Ganga (Narora)		River Ganga (Kanpur)		River Yamuna (Firozabad)		River Gomti (Lucknow)		Wilks lambda	F	*P* value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.			
1-2	1.27	0.17	1.25	0.16	0.96	0.19	1.03	0.19	0.649	36.09	0.000*
1-3	2.66	0.23	2.65	0.15	2.54	0.13	2.51	0.26	0.948	7.75	0.000*
1-4	2.62	0.17	2.68	0.28	2.46	0.19	2.43	0.30	0.842	12.46	0.000*
2-3	2.14	0.24	2.10	0.27	2.04	0.15	2.05	0.25	0.973	1.85	0.140
2-4	1.64	0.17	1.66	0.19	1.55	0.18	1.50	0.25	0.896	3.65	0.014
3-4	1.75	0.14	1.69	0.17	1.65	0.17	1.50	0.16	0.748	22.47	0.000*
3-5	2.72	0.31	3.06	0.25	2.54	0.19	2.75	0.39	0.728	24.90	0.000*
3-6	4.18	0.31	4.27	0.46	3.23	0.20	3.80	0.55	0.563	51.80	0.000*
4-5	3.82	0.38	3.99	0.36	3.08	0.17	3.35	0.49	0.557	53.05	0.000*
4-6	3.15	0.28	3.66	0.39	2.89	0.27	2.98	0.48	0.630	39.09	0.000*
5-6	2.97	0.24	3.01	0.43	2.21	0.13	2.52	0.42	0.593	45.76	0.000*
5-7	0.99	0.16	0.89	0.10	0.67	0.15	0.82	0.16	0.735	24.08	0.000*
5-8	3.53	0.27	3.34	0.26	2.66	0.13	3.05	0.39	0.593	45.72	0.000*
6-7	2.66	0.26	3.00	0.38	2.29	0.15	2.54	0.32	0.574	49.42	0.000*
6-8	1.34	0.19	1.41	0.18	1.42	0.13	1.20	0.21	0.776	19.25	0.000*
7-8	2.68	0.31	3.02	0.32	2.37	0.14	2.69	0.34	0.630	39.19	0.000*
7-9	12.34	2.01	13.30	1.71	8.29	0.41	9.31	0.47	0.332	134.01	0.000*
7-10	12.04	1.98	13.01	1.69	8.06	0.40	9.12	0.50	0.335	132.46	0.000*
8-9	11.90	1.84	12.43	1.43	7.92	0.44	9.08	0.55	0.323	139.73	0.000*
8-10	11.31	1.70	11.88	1.35	7.56	0.44	8.71	0.57	0.318	142.76	0.000*
9-10	1.18	0.12	1.12	0.13	1.08	0.10	1.04	0.14	0.855	11.31	0.000*
9-11	1.09	0.15	1.00	0.14	1.01	0.08	0.93	0.13	0.816	15.03	0.000*
10-11	0.34	0.06	0.34	0.05	0.50	0.03	0.29	0.06	0.292	161.96	0.000*
HL	2.79	0.12	2.72	0.14	2.51	0.13	2.49	0.29	0.815	15.14	0.000*
BD	2.85	0.20	2.90	0.26	2.30	0.11	2.66	0.38	0.624	40.16	0.000*
POL	1.67	0.10	1.64	0.21	1.60	0.12	1.63	0.19	0.983	1.18	0.319
ED	0.54	0.07	0.49	0.06	0.38	0.06	0.32	0.09	0.433	87.25	0.000*
PFL	1.88	0.09	1.94	0.14	1.85	0.11	1.85	0.23	0.951	3.43	0.018
VFL	1.46	0.12	1.41	0.10	1.31	0.10	1.33	0.18	0.844	12.34	0.000*

*$P < 0.001$.

a Scotian shelf population. In the present study, a decreased head length and eye diameter were observed in the Gomti River population which may have been associated with the presence of high turbidity in the Gomti River, as reported by Singh and Tondon (2010). Moore (1950) described that fishes living in turbid water adapted with a reduced eye diameter. In corroboration with the present findings on *H. fossilis*, Khan et al. (2012) also observed a smaller eye diameter in a *Channa punctatus* population from the Gomti River compared to populations of Ganga and Yamuna Rivers.

The presence of different stocks of *H. fossilis* at different sites of the 3 rivers may be attributed to geographical isolation and environmental parameters which could have hindered the movement of fish from intermingling with populations in other rivers. The Ganga, Yamuna, and Gomti Rivers originated from different sources. The sampling stations on these rivers were isolated from each other. However, both the Yamuna and Gomti Rivers are tributaries of the Ganga River, and both meet the Ganga River approximately 750 and 350 km downstream, from the fish sample collection sites. Therefore, it can be assumed that these populations are geographically isolated to a large extent; with only a chance of intermingling where the Yamuna and Gomti Rivers merge into the Ganga River. Differences in the results of morphometry and otolith chemistry might have been due to regional differences in the fish's habitats which suggests that the groups of fish have spent the major part of their lives in different regions and consequently showed stock separation. It appears plausible that *H. fossilis* populations from the 2 sites on the Ganga River could have been produced out of a common fish stock, thereby showing no stock discrimination using truss morphometry. However, otolith chemistry results indicated that the fish populations from these 2 sites were experiencing entirely different habitat qualities, which were sufficient to

Table 4. Principal component loadings for truss morphometric characters of *H. fossilis*

	Principal Components		
Characters	PC I	PC II	PC III
1-2	-0.935	0.269	-0.109
1-3	0.948	0.292	-0.086
1-4	0.171	0.370	-0.162
2-3	0.226	0.432	-0.275
2-4	-0.059	0.143	-0.106
3-4	0.085	0.657	-0.275
3-5	0.786	0.131	-0.126
3-6	0.920	0.120	-0.102
4-5	0.935	-0.189	0.021
4-6	0.795	0.099	-0.112
5-6	0.936	-0.189	0.023
5-7	-0.493	0.372	-0.217
5-8	0.892	0.286	-0.090
6-7	0.806	0.376	-0.090
6-8	0.003	0.523	-0.273
7-8	0.844	0.315	-0.076
7-9	0.560	0.219	-0.167
7-10	0.515	0.433	-0.114
8-9	0.532	0.215	0.620
8-10	0.270	0.410	0.692
9-10	-0.233	0.695	-0.126
9-11	-0.207	0.660	-0.113
10-11	-0.623	0.313	-0.266
HL	0.962	-0.176	0.033
BD	0.925	-0.182	0.036
POL	-0.224	0.416	0.535
ED	-0.981	0.299	-0.062
PFL	-0.036	0.36	0.678
VFL	-0.138	0.455	0.681
Percent of variance explained	39.1	13.4	10.1

Table 5. Results of a discriminant function analysis (DFA) showing the number and percentage of individuals classified into each group for morphometric characters from the original matrix

	River Ganga (Narora)	River Ganga (Kanpur)	River Yamuna (Firozabad)	River Gomti (Lucknow)	Total
River Ganga (Narora)	28	10	1	1	40
%	70	25	2.5	2.5	100
River Ganga (Kanpur)	15	27	4	4	50
%	30	54	8	8	100
River Yamuna (Firozabad)	1	4	40	3	48
%	2	8.3	83.3	6.3	100
River Gomti (Lucknow)	1	5	6	54	66
%	1.5	7.6	9.1	81.8	100

produce variations in the elemental profiles of their otoliths. Since geographic and climatic variations at the 2 sampling sites of the Ganga River are not so pronounced, water quality appears to be one of the major factors discriminating *H. fossilis* populations at the 2 sites on this river.

Elemental concentrations in the habitat were suggested to greatly influence the uptake and incorporation of elements in fish otoliths. It was assumed that various factors may control the uptake and incorporation of elements into otoliths such as temperature (Fowler et al. 1995, Elsdon and Gillanders 2004, Walther et al. 2010), salinity (Fowler et al. 1995), water chemistry (Bath et al. 2000, Elsdon and Gillanders 2003 2004), growth rate (Kalish 1989), diet (Buckel et al. 2004), ontogeny (Walther et al. 2010), stress (Kalish 1992), and physiology (Kalish 1989), although the relative influence of these factors remains poorly understood for most species. It may be assumed that morphological differences within a species are produced by the combined interactive effects of the environment, selection, and genetics on individual phylogenies as a consequence of geographical isolation which can lead to the development of different morphological features among fish populations (Cadrin 2000, Poulet et al. 2005). Further, it was reported that biotic and abiotic conditions induce the formation of specific characteristics of an individual (Bailey 1997) that later form the basis of definition of different stocks.

Begg and Waldman (1999) suggested that the use of more than 1 stock identification methods and comparison between them can increase the likelihood of correctly classifying stocks. The high allocation success of trace elements might be due to the fact that the elemental fingerprint reflects the lifelong exposure of individual fish to both the environment and its own physiology, and thus would be expected to differ among groups of fish which have experienced different histories, whether or not the groups come from the same population (Campana et al. 2000). Turan (2006), Longmore et al. (2010), and Ferguson et al. (2011) reported that otolith chemistry was the most efficient tool for discriminating the fish stocks compared to otolith shape.

Despite the high allocation success, otolith chemistry is an expensive and time-consuming technique requiring intensive care during the decontamination process and sample preparation; even a slight contamination can change the result (Campana 1999). A truss network analysis can potentially be used as a cheap, accurate, and precise method for identifying discrete stocks; however it showed differentiation of discrete stocks with low classification success (72.27%) compared to the trace elements analysis.

It was concluded that (i) analysis of the elemental profile of otoliths is a more-powerful and accurate tool than truss morphometry for delineating stocks of *H. fossilis*, and (ii) fish collected from different sites in the present study belonged to different stocks. Further, it was noted that truss morphometry provides a cost-effective method with sufficient discriminatory power for many management decisions.

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