FULL PAPER



Otolith fingerprints reveal stock discrimination of *Sperata* seenghala inhabiting the Gangetic river system

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Abstract Variations in elemental concentrations of otoliths were used to study the probable stock(s) of Sperata seenghala in the Gangetic river system. Fifteen trace elements from whole sagittal otoliths were analysed using inductively coupled plasma-atomic emission spectrometry. Strontium, barium, lithium, copper, iron, lead, zinc, manganese, nickel (P < 0.001) and magnesium (P < 0.01)differed significantly among locations, while no significant differences were noted for calcium, sodium and potassium (P > 0.01). Chromium and cadmium were not detected in the otoliths of the fish from Narora site on the river Ganga. Discriminant function analysis using cross-validation classification assigned individuals to their site of sampling origin with a mean classification accuracy of 83.2 %. The detected site-specific elemental differences in S. seenghala otoliths indicate a high level of site-fidelity in relation to their habitat areas. Thus target fish population from these sites can be regarded as separate stocks.

Keywords Otolith fingerprints · *Sperata seenghala* · Stock discrimination

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Introduction

A fish stock may be defined as an intra-specific group of randomly mating individuals with temporal or spatial integrity (Ihssen et al. 1981). Identification of intraspecific groups with variable life history attributes is considered necessary for understanding population dynamics and the evaluation of sustainable harvests (Cadrin 2000). Understanding the distribution and movement of the target fish species can lead to a better understanding of connectivity, philopatry, critical habitats or specific life history events that may improve fitness, which ultimately will structure populations. Such information can, in turn, be used to guide rational exploitation and conservation strategies. Over the years and more particularly during the recent past, the fish population has shown declining trend in the rivers, Ganga, Yamuna, Krishna, Sutlej, Ravi, Beas, Narmada, Tapi, Mahi, Sabarmati, Pennar, Cauvery, Betwa and Gomti because of the destruction of fish habitat by alteration of river beds, increased water abstraction, overfishing, pollution of fishery waters, indiscriminate land development and domestication of species, introduction of non-native species, global climatic variation and construction of dams, etc. (Lakra et al. 2010; Dandekar 2012).

Otoliths are innate data archives that document information in their microstructure and chemistry at different spatial and temporal scales related to their growth and environment. This information, which includes age and growth, movement patterns and habitat interactions, can be interpreted at the population level in terms of ecology, demography and life history of the species, which has become fundamental to the management of fisheries and protected species around the world. One of the most appreciated characteristics of the otoliths is being metabolically inert and showing lack of resorption

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(Campana and Neilson 1985; Kalish 1989; Campana 1999; Miyan and Khan 2014). This means that once the material has been deposited, the organism will not use it again even in periods of starvation. Elemental composition of otoliths has been effectively utilized for the stock identification of various freshwater fish species such as *Oncorhynchus clarki lewisi* (see Wells et al. 2003), *Perca flavescens* (see Brazner et al. 2004), *Morone saxatilis* (see Schaffler and Winkelman 2008) *Heteropneustes fossilis* (see Khan et al. 2012), *Channa punctata* (see Miyan et al. 2014), *Wallago attu* (see Miyan 2014) and *Clarias batrachus* (see Miyan et al. 2015) etc.

Sperata seenghala is an economically important species having good food value in different south Asian countries of its distribution range and particularly in Indian sub-continent. It usually inhabits the freshwaters such as rivers, lakes, reservoirs, floodplains, and tanks with mud or silt laden bottoms and grasses (Talwar and Jhingran 1991). It is widely distributed in India, Pakistan, Bangladesh, Sri Lanka, Nepal, Burma, Thailand, Vietnam, Kampuchea, Malay Peninsula, Indonesia and Afghanistan (Talwar and Jhingran 1991; Froese and Pauly 2014). The adult fish is column feeder and carnivorous in nature and consumes a variety of living creatures including fish, frogs, snakes, insects, prawn, earthworms, tadpoles, crustaceans, molluscs and debris. Juveniles are mainly bottom and marginal feeders; feed mainly on fish fry and fingerlings of other fishes, prawns, shrimps, insects, crustacean, rarely plant matter including debris (Gupta 2015). Fish breed during April to August in Indian rivers, Bangladesh and Pakistan (Bhatt 1970; Gupta 2015). Fecundity of this fish varies from 13,005 to 119,943 eggs (Rahman et al. 2014). Current field surveys showed that it is still relatively abundant and categorized as "Least Concern" (IUCN 2015). In India, the necessary basic biological information particularly that related to stock identification of S. seenghala inhabiting major habitats such as the Gangetic river system is still warranted. Such information is considered essential to understand the characteristics and dynamics of a population which may be of great help in devising the strategies for maintaining the genetic diversity, conservation and rational exploitation of the target species.

There is lack of published information on the stock structure of *S. seenghala* from the selected river system. However, few reports are available on genetic variability of *S. seenghala* from other rivers such as Sutlej and Beas (Saini et al 2010) and Bhadbada reservoir, Mohinisagar reservoir, Bansagar reservoir, Bargi reservoir and Gandhisagar reservoir in Madhya Pradesh, India (Garg et al. 2014). The present study was, therefore, undertaken to investigate the stock structure of *S. seenghala* population inhabiting the river Ganga and its tributaries: river Yamuna and river Gomti using variation in elemental composition of otoliths.

Materials and methods

Study area. Samples of Sperata seenghala were collected from three rivers in northern India, the Ganga, Yamuna and Gomti. The river Ganga originates in the Garhwal Himalayas $(30^{\circ}55' \text{ N}, 70^{\circ}07' \text{ E})$ at an elevation of 4,100 m above mean sea level from Gaumukh glacier in the western Himalayas in the Uttarakhand state of India. It flows about 2,525 km before falling into the sea (Kamyotra 2009) and finally drains into the Sundarban delta in the Bay of Bengal. The river Ganga harbours about 265 fish species of which 34 including the prized Gangetic carps (Labeo rohita, Catla catla, Cirrhinus mrigala and Labeo calbasu), large catfishes (Sperata aor, Sperata seenghala, Wallago attu, Bagarius bagarius), featherbacks (Notopterus notopterus, Notopterus chitala) and murrels (Channa marulius, Channa punctata) are of great commercial interest (Sinha and Khan 2001). The river Yamuna originates from the Yamunotri glacier (Saptrishi Kund) near Bander punch peaks (38°59' N, 78°27' E) at an elevation of circa 6,320 m above mean sea level in the Mussoorie range of the lower Himalayas in Uttarkashi district of Uttarakhand, India, and traverses some 1,336 km through five states before finally merging into the river Ganga in Allahabad, Uttar Pradesh, India (Sengupta 2006). It is the largest tributary of river Ganga. Choudhury et al. (2002) reported 87 fish species from the Okhla bird sanctuary site on the river Yamuna. The river Gomti originates from a natural lake (28°34' N, 80°07' E) Gomat Taal near Madho Tanda, Pilibhit town in Uttar Pradesh, India, about 50 km south of the Himalayan foothills (Sarkar et al. 2010). It travels about 750 km to finally merge into the river Ganga near Saidpur Kaithi in Ghazipur district bordering Varanasi district in Uttar Pradesh, India (Sarkar et al. 2010). Sarkar et al. (2010) have documented 56 fish species belonging to 20 families and 42 genera from the river Gomti. The authors further argued that the fish fauna of the river Gomti is adversely affected due to human induced threats like sewage pollution, habitat alteration, etc.

Sample collection. Fish samples of *S. seenghala* were collected during September 2012 to January 2013 from Narora located at 27°30' N, 78°25' E [n = 25; mean standard length and standard deviation, MSL \pm SD (cm) = 41.47 \pm 2.33], Kanpur located at 26°28' N, 80°24' E [n = 25, MSL \pm SD (cm) = 40.18 \pm 3.29] and Bha-galpur located at 25°16' N, 87°01' E [n = 25, MSL \pm SD (cm) = 41.12 \pm 2.89] on the river Ganga, Firozabad site located at 27°09' N, 78°24' E [n = 25, MSL \pm SD (cm) = 40.68 \pm 2.98] on the river Yamuna and Lucknow site located at 26°55' N, 80°59' E [n = 25, MSL \pm SD (cm) = 39.91 \pm 2.62] on the river Gomti (Fig. 1).

Otolith removal and decontamination. The sagittal otoliths were removed from otic capsules by opening the otic bulla. The otoliths were collected in plastic vials, brought to the laboratory and stored in eppendorf tubes. Otoliths were taken from the fishes of similar lengths to include the fishes of same cohort. Prior to decontamination of otoliths, all implements and glasswares were cleaned with analytical grade 1 % nitric acid (HNO₃). We have taken left sagittal otoliths for elemental analysis. We kept right sagittal otolith for age estimation of the fish. All the adhering biological residues were removed by putting the otoliths in ultra pure water overnight. To remove surface contamination, otoliths were soaked in 3 % hydrogen peroxide for 5 min and immersed for 5 min in 1 % HNO₃. Otoliths were then flooded with ultra-pure water for 5 min to remove the acid. After decontamination, the otoliths were dried under a laminar flow hood and weighed to the nearest 0.1 mg (Turan 2006; Khan et al. 2012).

Sample preparation and elemental analysis. The decontaminated otoliths were dissolved in 10 ml of 37 % HNO₃ and the volume was brought up to 25 ml with Milli Q water. Inductively coupled plasma–atomic emission spectrometry (ICP–AES) (Thermo Electron IRIS Intrepid II XSP DUO) was used to analyse the elements (Ca, Na, Mg, Sr, Ba, Mn, Fe, Cu, Pb, Ni, Zn, Li, Cr, Cd and K) in the otoliths. Blank samples were prepared similarly but without the otolith and were used to correct for background noise in readings and to calculate detection limits. Internal standards Ga and In were added in samples and blanks which were used to correct for the remaining

matrix effect and to compensate instrument drift. For external calibration, multi-elemental standards were prepared with high purity ICP multi-element standard solution IV certiPUR (NIST SRM) obtained from Merck (Germany) using ultra pure Milli Q water and 2 % v/v HNO₃ analytical grade. A calibration blank was also prepared in similar manner. The calibration curve was obtained for five points. The concentration of elements in the sample and blank were calculated and expressed as microgram per gram on dry weight basis. To minimize the possibility of contamination, all samples, standards and blanks were prepared in a laminar flow hood (Turan 2006; Khan et al. 2012).

Data analysis. To check the effects of the standard length (SL) on elemental concentrations, correlations were carried out between elemental concentrations and the SL of fish. Significant correlations were observed between the fish size and elemental concentrations of samples. The data were standardized according to Bergenius et al. (2005) to remove the effect of the SL from each elemental concentration:

$$C_{ij adj} = C_{ij} \pm b(L_{Sij} - L_{SMi})$$

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_{Sij} common to all groups, L_{Sij} is the SL of fish i in group j and L_{SMi} is the average SL in group j.



Fig. 1 Collection sites of *Sperata seenghala*

Data were tested for normality using Shapiro-Wilk test. Levene's test and homogeneity of the group covariance matrix by Box's M test (MANOVA) was used to examine the assumption of homogeneity of variance for each dependent variable. The adjusted concentrations for all elements of interest were then analysed by a multivariate analysis of variance (MANOVA) to test for spatial differences in the multivariate elemental chemistry. Wilk's λ criterion was used to test for group differences in the MANOVAs. A univariate ANOVA was used to compare the mean elemental concentrations of otoliths among different sites of the rivers. Elemental concentrations of otoliths were subjected to post hoc test (Tukey's) to assess significant differences in the fish otoliths from the different collection sites. A stepwise discriminant function analysis (DFA) was used to examine the elemental chemistry in discriminating populations among the sites and to investigate whether elements could be used to classify samples into their original group. A leave-one-out classification with cross-validation was carried out to assign individuals to their original group. The scatterplots of first two discriminant scores were drawn to depict the separation of stocks on the graph.

All the statistical analyses were carried out on MS-EXCEL (Microsoft Corporation, Redmond, WA, USA) and SPSS vers. 16 (SPSS, Chicago, IL, USA).

Results

Differences in mean elemental concentrations of otoliths among sites were significant (MANOVA, P < 0.001). Strontium, barium, lithium, copper, iron, lead, zinc, manganese, nickel (ANOVA, P < 0.001) and magnesium (ANOVA, P < 0.01) differed significantly among locations, while no significant differences were noted for calcium, sodium and potassium (ANOVA, P > 0.01) (Table 1). Thus, calcium, sodium and potassium were removed from further analysis. Chromium and cadmium were excluded from the final analysis since these elements were not detected in the otoliths of the fish from Narora site on the river Ganga. Otoliths of fish collected from Kanpur site on the river Ganga exhibited significantly (ANOVA, P < 0.001) higher values of Sr, Li and Zn compared to fish otoliths from other sites. However, Ca, Na and K exhibited comparable (ANOVA, P > 0.01) values among different sites. Ba was significantly higher (ANOVA, P < 0.001) in otoliths of fish from Narora site on the river Ganga. Mn, Cu and Fe were significantly higher (ANOVA, P < 0.001) in otoliths of the fish collected from Bhagalpur site on the river Ganga. A significantly higher (ANOVA, P < 0.001) concentration of Pb was found in otoliths of the fish from Firozabad site on the river Yamuna. Mean elemental

Table 1 ANOVA results for the elemental concentration for otoliths

 of Sperata seenghala collected from the Ganga river and its tribu

 taries: the Yamuna and Gomti Rivers

Elements	Wilks' λ	F	df1	df2	Sig.
Ca	0.908	3.052	4	120	0.020
Na	0.961	1.223	4	120	0.305
Mg	0.883	3.962	4	120	0.005**
Mn	0.463	34.835	4	120	0.000*
Sr	0.631	17.555	4	120	0.000*
Cu	0.287	74.665	4	120	0.000*
Fe	0.561	23.456	4	120	0.000*
Li	0.462	34.915	4	120	0.000*
K	0.929	2.295	4	120	0.063
Pb	0.631	17.534	4	120	0.000*
Zn	0.524	27.207	4	120	0.000*
Ni	0.240	94.801	4	120	0.000*
Ba	0.414	42.418	4	120	0.000*

* *P* < 0.001, ** *P* < 0.01

concentration of Ni was significantly (ANOVA, P < 0.001) higher in otoliths of the fish collected from Lucknow on the river Gomti (Fig. 2). The Wilks' λ test of discriminant function analysis showed significant differences in otolith elemental concentration of all the populations (P < 0.001) (Table 2). The first discriminant function (DF I) accounted for 63.5 % of the total variation. DF II and DF III accounted for 24.1 % and 9.6 %, respectively, of the group variability among the populations while DF IV accounted for 2.8 % variation. The elements Cu, Ba and Fe contributed most in discriminating among the populations in DF I, the elements Ni and Zn contributed to DF II, and Sr and Mn contributed to DF III in discriminating the populations (Table 3). DF I and DF II accounted for 87.6 % of the group variability among the population. DF I vs. DF II graph depicted the presence of five distinct units and showed clear separated stocks (Fig. 3). DFA using cross-validation classification was able to discriminate fish among original locations with high degree of accuracy: Lucknow (92 %), Firozabad (88 %), Bhagalpur (84 %), Kanpur (80 %) and Narora (72 %). A total of 83.2 % fishes were correctly classified to their site of capture (Table 4).

Discussion

Otoliths are now becoming an important tool serving as invaluable resource for fisheries science (Campana 2005). The deposition of trace elements into otolith structure is a multicomplex procedure which is still not fully studied and understood, but we assume that several abiotic (e.g. salinity Fig. 2 Mean elemental concentrations and standard error for otoliths of *Sperata seenghala*; bars having similar letters are insignificantly different (P > 0.05); Cr and Cd were below the detection limit in the fish otoliths collected from Narora site of the river Ganga



Table 2 Wilks' λ test for verifying differences among stocks of *Sperata seenghala* with otolith chemistry using DFA

5.6 4.8

3.2-2.4-1.6-0.8-

Narora

Test of function(s)	Wilks' λ	χ-square	df	Р
1 through 4	0.009	555.088	40	0.000*
2 through 4	0.079	296.079	27	0.000*
3 through 4	0.325	131.013	16	0.000*
4	0.730	36.662	7	0.000*

* P < 0.001

 Table 3 Contribution of otolith chemistry to the discriminant functions for Sperata seenghala

Elements	DF1	DFII	DFIII	DFIV	
Cu	0.514*	0.018	-0.495	-0.090	
Ba	-0.412*	0.064	0.026	-0.074	
Fe	0.325*	0.024	-0.220	-0.052	
Ni	0.218	-0.929*	0.241	0.047	
Zn	0.270	0.393*	0.186	-0.111	
Sr	0.226	0.123	0.662*	0.027	
Mn	0.069	0.059	0.404*	-0.181	
Pb	0.314	-0.124	-0.335	0.532*	
Li	0.126	0.039	0.525	-0.485*	
Mg	0.336	0.173	0.205	0.237*	

* Largest correlation between each variable and any DF

and temperature) and biotic (e.g. feeding regimes, metabolic rates, ontogenetic events, and vital effects such as, age, growth rate, gonad maturation) factors are responsible



Fig. 3 Scatterplot of the first two canonical discriminant scores from the DFA for otolith chemistry

to control the rate and uptake of elemental incorporation into otoliths (Bath et al. 2000; Milton and Chenery 2001; Gillanders and Kingsford 2003; Sturrock et al. 2012). Univariate ANOVA showed that the mean elemental concentrations obtained in the whole otolith analysis, namely Sr, Ba, Mg, Li, Cu, Fe, Pb, Zn, Mn, Ni and Mg showed significant differences among the five sites of three rivers. In addition, the concentrations of Sr, Li, Zn and Mg were higher in the otoliths of the fish collected from Kanpur site on the river Ganga. One plausible reason for this may be **Table 4** Classification matrixof Sperata seenghala specimensbased on whole otolithelemental concentrations usedin DFA

Sampling sites	Narora	Kanpur	Bhagalpur	Firozabad	Lucknow	Total
Narora	18	4	2	1	0	25
%	72	16	8	4	0	100
Kanpur	0	20	3	1	1	25
%	0	80	12	4	4	100
Bhagalpur	0	2	21	1	1	25
%	0	8	84	4	4	100
Firozabad	0	1	2	22	0	25
%	0	4	8	88	0	100
Lucknow	0	0	2	0	23	25
%	0	0	8	0	92	100

the presence of the higher concentrations of these elements in the ambient environment of the sampling site (Purushothaman and Chakrapani 2007; Kamyotra 2009; Gowd et al. 2010). Kanpur is the hub of a large number of industries, viz; plastics, leather, textiles, a thermal power plant, cement, fertilizers, ordnance, two wheeler automobiles, electronic products, etc. The effluents of these industries drain into river Ganga, contributing to a greater abundance of trace elements in the ambient water of the river Ganga at Kanpur site and probably in otoliths of the fishes caught from Kanpur site. Gowd et al. (2010) reported that soil in the river Ganga at Jajmau site (Kanpur) is heavily contaminated with Sr along with various heavy metals, which could have been incorporated into the river water from surface runoff and also suggested that the higher concentration of strontium might be due to the water contamination resulting from industrial effluents in adjoining areas. Otoliths of the fish collected at the Narora site on the river Ganga showed highest concentration of Ba. This may be due to relatively better water quality at Narora site as it lies on the upper stretch of the river Ganga (Purushothaman and Chakrapani 2007; Kamyotra 2009). The source of Ba in the river Ganga is the Himalayan rocks. It was observed that mobility of Ba decreased compared to other elements in the downstream stretch (Das and Krishnaswami 2006). Highest concentration of Pb was detected in the otoliths of fish sampled at Firozabad site on the river Yamuna. This may be due to large number of glass industries pouring effluents into the river Yamuna at Firozabad. High levels of organic contents, nutrients, heavy metals (Cd, Cr, Ni, Pb, Fe, Cu and Zn) and pesticides have been reported from river Yamuna (Sengupta 2006). Highest concentration of Mn, Cu and Fe was detected in the otoliths of fish sampled at Bhagalpur site on the river Ganga. Several studies have shown that higher levels of heavy metals in otoliths are in accordance with an environmental exposure history of fish to aquatic contamination (Geffen et al. 2003; Ranaldi and Gagnon 2010). The DFA showed highly significant divergence among the populations of Sperata seenghala. It is assumed that if different fish populations inhabit different aquatic environments, or at least have a very prolonged exposure to different water environments, the otolith elemental composition should serve as a natural tag for these groups (Campana 1999). This could be due to the characteristic of fish otoliths to retain permanent record of the elements incorporated onto its growing surface and being chiefly regulated by the physico-chemical environment in which fish lives. Differences in habitat quality due to geographical separation of the sampling sites could have resulted in different elemental concentrations of otoliths of the fish collected from different sites not only in different rivers but also at three sampling sites within the river Ganga. It was assumed that various factors 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 2004), growth rate (Kalish 1989), diet (Buckel et al. 2004), ontogeny (Walther et al. 2010), stress (Kalish 1992), genetics (Clarke et al. 2011) and physiology (Kalish 1989) may control the uptake and incorporation of elements into otoliths. However, the mechanism of incorporation of elements in the otoliths is poorly understood for most of the fish species (Clarke et al. 2011) and has also not been investigated in the present study.

The multi-elemental analysis of whole otolith composition using DFA showed that the elements Cu, Ba, Fe, Ni, Zn, Sr, Mn, Pb and Li are the potent discriminator of target species stocks in the study area. Multi-elemental concentrations obtained for each site showed a good trace-back result to the original location areas (83.2 % mean correct classification) suggesting a limited movement among the areas where fishes were captured. The incorrect classification (16.8 %) may be due to the movement of fish for the feeding/breeding purpose (Gupta 2015). Mixing of fishes was observed more between the sites, which are near to each other viz, Narora and Kanpur sites on the river Ganga. A lesser misclassification observed in the fish from river Gomti could be because of the fact that this tributary joins the river Ganga at a place downstream to which only one sampling site, Bhagalpur is located. The extent of potamodromous migration (Froese and Pauly 2014) of S. seenghala is not well studied. Although it seems probable that the fish is not highly migratory and the migration occurs presumably for the purpose of acquiring food. Furthermore, the movement of fish is inhibited by the Dams constructed at Narora and Kanpur on the river Ganga that could have consequently led to the stock separation in the river Ganga. Our results showed that elemental concentrations of whole otoliths are site-specific and that they can provide natural tags of their feeding/growing areas. The high classification success (92 %, 88 %, 84 %, 80 % and 72 % to Lucknow, Firozabad, Bhagalpur, Kanpur and Narora, respectively) implied that S. seenghala shows fidelity to its feeding/growing area. Similar variation trends in the elemental composition of otoliths were obtained for the stinging catfish, Heteropneustes fossilis (see Khan et al. 2012) and Channa punctata (see Miyan et al. 2014) population from the same habitats of the Ganga river system.

It may be concluded that the *S. seenghala* populations collected from different sites of the Gangetic river system may be categorized as separate stocks based on the variation in the elemental profile of otoliths. For validation and better inference of the present findings, further studies are warranted on the elemental analysis in environmental water of each collection site of three studied rivers.

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