DOI: https://doi.org/10.36094/sc.v91.2025.Delineating_the_Spatio_Temporal_Variation_in_Otolith.Borah.224

ARTICLE

*Sci. and Cult.* 91 (3–4) : 224-232 (2025)

# DELINEATING THE SPATIO-TEMPORAL VARIATION IN OTOLITH MICROCHEMISTRY OF MIGRATORY *TENUALOSA ILISHA* (HAMILTON, 1822) FROM BRAHMAPUTRA RIVER, INDIA

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*This study explores spatial and temporal variations in trace element composition in otoliths of Hilsa (Tenualosa ilisha) from two landing sites along the Brahmaputra River in Assam, India- Guwahati and Dhubri. Otoliths from 60 fish were analyzed for 14 trace elements using ICP-MS. Results showed significant spatial variation, with Chromium and Zinc dominant in Guwahati, and Copper in Dhubri. Statistical analysis indicated high classification accuracy (93.33%) in distinguishing populations. The findings highlight the use of otolith microchemistry for migratory pattern analysis and sustainable fisheries management.*

***Keywords:*** *Sagittal Otolith, Trace Elements, ICP-MS, Stock Characterization*

## *Introduction*

Brahmaputra and Barak, along with their 53 tributaries are two major river systems of Assam and north-east India. A major transboundary river, Brahmaputra, drains Tibet, north-east India and Bangladesh. Originating at 5300 masl in the glacial mass of Kailash Range in the mighty Himalayas the river flows by the name of Yarlung-Tsangpo for a distance of 1,625 km in Tibet and enters India as Siang at Gelling near Tuting in Upper Siang district of Arunachal Pradesh. Siang or Dihang as the Brahmaputra is known in Arunachal Pradesh flows for a distance of 278 km through the state. The river then enters Assam and is joined by Dibang and Lohit, two large Himalayan rivers and the river flows by the name of Brahmaputra through the length of state of Assam from Sadiya to Dhubri traversing a distance of 640 km. After travelling for a distance of 918 km in India, Brahmaputra enters Bangladesh, where it is popular by the name of Jamuna, where it flows for another 337 km[1].

Around 200 fish species are available in Brahmaputra and Irrawaddy River systems[2]. In the recent studies, a total of 141 finfish species were reported under 84 genera and 29 families from Brahmaputra River stretch in Assam. Besides finfishes, the river stretch in Assam also harbours a rich diversity of shellfishes[1]. Among the shellfishes, the two freshwater prawns *Macrobrachium gangeticum* and *M. malcolmsonii* formed fisheries of commercial importance in the river stretch, with *M. gangeticum* being more abundant[1].

Diversity in topography, particularly gradient and variation in climatic pattern, fish species structure and

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abundance exhibit variation along the stretch of the river in Assam. Finfishes and shellfishes of commercial importance includes the Indian major carps, minor carps, cat fishes, feather backs, Hilsa (*Tenualosa ilisha*), miscellaneous species like *Aspidoparia morar*[3], *Puntius* spp. etc. and freshwater prawns are of common occurrence in the river. Medium carps like *Labeo gonius*, minor carps like *L. bata*, catfishes like *Wallago attu* and feather backs like *Notopterus notopterus* are dominant in the upper stretch of the river from Sadiya to Dibrugarh, along with major carps. Along the lower stretch of Brahmaputra in Assam, which lies till Dhubri, Indian major caprs, cat fishes, minor carps and the migratory *T. ilisha* are common in occurrence[1]. *T. ilisha,* a highly priced commercial species exhibits considerable growth[4], unique reproductive behaviours[5] and feeding[6] behaviours in its diverse habitats. About 70% of hilsa landing in India comes from the marine and inland waters of the east coast compared to 30% from west coast waters[7].

The past few decades has witnessed major technological advances in the field of fisheries science in general and study of otoliths in particular, with developments in deriving information from fish otolith[8-12]. Otoltihs are acting as natural log books for researchers working the field of fisheries science as they record valuable and reliable information on the growth and life history of fishes at a spatial and temporal scale[13,14].

To carry out stock identification, one essential requirement is to identify the origin of fish, which is a difficult task in fresh, brackish and marine ecosystems[15,16]. A reliable answer to this problem is the use of trace element concentration in otolith of fish species[14,17,18] and more applicable in the case of long distance migratory fishes like Hilsa. Even though stock characterization studies based on morphometric characters has been carried out from different geographical locations of India[19], studies on otolith microchemical composition of Hilsa are rare from Indian waters. However authors have studied the relationship between otolith dimensions and fish body size[20]. Otolith in fishes is basically calcium carbonate ($CaCO_3$). However, certain trace elements like Strontium (Sr), Barium (Ba) etc. is also found in otolith of fishes, concentration of which is a direct reflection of their presence in the environment[21-23] and can help researchers in tracing migratory route of fishes. Otoliths in fishes are chemically inert and hence the concentration of trace elements can help researchers in arriving at conclusions regarding the environment in which the individual fish species or group resided on a sequential manner[11].

Trace element concentration in otoliths has been successfully used in detecting variation in fish species on a spatial scale[24-26]. Temporal variability in trace element composition in otoliths is also demonstrated by researchers over the years[25,27]. It was observed that significant variation exists in otolith trace element composition of the highly migratory Atlantic cod, *Gadus morhua*, collected from Fundyrip and Georges Bank, Atlantic Ocean[28]. It was observed that trace element composition in otolith of *T. toli* collected from Bangladesh waters varied across years, which he attributed to changes in water chemistry[29]. Analysis of the trace element composition of Hilsa otoliths from Bangladesh waters was done during 1996 and 1997[30]. However, such studies on otolith micro-chemistry of Hilsa from Indian waters are quite limited and same is the case for Indian part of Brahmaputra River system and hence the present study holds great importance.

## Materials and Methods

***Study Area, Study Period and Sample Collection:*** *T. ilisha* specimens were collected from in Uzanbazar, Guwahati (26°11'44.33'' N and 91°45'23.94'' E) and Shri Ramghat, Dhubri (26°0'36.50'' N and 89°58'41.61'' E) landing centres of Brahmaputra River (Fig. 1). Sampling was carried out in October, 2017, October, 2018 and March, 2019 in Dhubri and Guwahati landing centres of River Brahmaputra. Fish were caught using drift gill nets of mesh size 30-80 mm. Specimens caught fresh were preserved in 10% neutral buffer formaldehyde and brought to Institute laboratory for analysis. The specimens were identified following standard manuals[31]. Sagittal otoliths from 60 specimens were collected for otolith micro-chemistry studies from Dhubri and Guwahati landing centres of River Brahmaputra in Assam.

***Analysis of Trace Element Concentration:*** To measure the trace elemental composition in otoliths, a total of 0.2 g otolith from frontal, mid and hind region was taken and dissolved in Nitric acid ($HNO_3$)[32]. Trace elements (n = 14) were measured using Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

***Statistical Analysis:*** Data on trace element concentration obtained from ICP-MS analysis of dissolved otolith sections were log transformed using MS Excel 2010 (Microsoft, 2010) and then subjected to discriminant function analysis[29] using Statistica 12.0 (StatSoft, 2013), to assess if any significant differences exist in trace elemental composition of Hilsa otoliths across time and space in Brahmaputra River, Assam.

## *Results*

Sagittal otolith (n=60) (Figure 2) was recovered from *T. ilisha* specimens collected from Guwahati, October 17 (Guwahati 2017); Dhubri, October 2017 (Dhubri 2017); Guwahati, October 2018 (Guwahati 2018); Dhubri, October 2018 (Dhubri 2018) and elemental composition for 14 different trace elements were analysed using ICPMS. With regard to morphometric measurements of otoliths extracted from *T. ilisha* specimens collected from River Brahmaputra, the total length of otolith was found to range from 4.10-6.12 mm with a mean value of 5.18±0.09 mm. Total weight of otolith ranged from 4.30-11.50 mg with a mean weight of 7.67±0.37 mg. Otolith width was found to range from 2.03-2.91 mm with mean value of 2.30±0.04 mm (Table 1).

**Table 1. Descriptive statistics of otolith size and weight measurements of *T. ilisha* from River Brahmaputra in Assam**

| Ecosystem | Sample size | Parameter | Min-Max | Mean±SE |
|---|---|---|---|---|
| River Brahmaputra | 60 | Otolith length (mm) | 4.10-6.12 | 5.18±0.09 |
| | | Otolith weight (mg) | 4.3-11.5 | 7.67±0.37 |
| | | Otolith width (mm) | 2.03-2.91 | 2.30±0.04 |

Min, Minimum; Max, Maximum; SE, Standard error

Mean concentration of 14 different trace elements in otolith of *T. ilisha* from four populations are given in Table 2. Based on the mean concentration it was seen that Chromium (Cr) was found to be most dominant trace element followed by Zinc (Zn) in Guwahati 2017 and Guwahati 2018 population of *T. ilisha*. The third dominant trace element was Gallium (Ga) in Guwahati 2017 population, while in Guwahati 2018 population it was Manganese (Mn). In case of Dhubri 2018 population Copper (Cu) was found to be most dominant among all 14 trace elements analysed, followed by Zn and Cr, while in Dhubri 2017 population, Zn was found to be most dominant, followed by Cu and Cr. Cadmium (Cd) was found to be the least dominant trace element for three populations *viz*. Guwahati 2018, Guwahati 2017 and Dhubri 2018, while Beryllium (Be) was found to have the least dominant among all 14 trace elements in Dhubri 2017 population. Details of correlation co-efficient of trace element concentration are given in Table 3 and Fig. 3.

Trace element concentration data was log transformed and then subjected to statistical analysis to find out if any significant differences exist among the four populations. Factor loadings (varimax rotation) based on principal component of concentration of 14 trace elements (log transformed) in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam shows that 10 elements namely, Lithium (Li), Beryllium (Be), Chromium (Cr), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Cadmium (Cd) and Lead (Pb) were loaded in Factor 1 (>0.70), while Manganese (Mn) showed significant loadings in Factor 2 (Table 4). Discriminant function analysis showed that out of the 11 trace elements, 10 trace elements showed good fitting to the model (Table 5), while Beryllium (Be) did not show a good fitting and was hence excluded. Mahalanobis distance was found to be on the lower side between Guwahati 2017 and Guwahati 2018 and between Dhubri 2017 and Dhubri 2018 populations, while it was found to be on the higher side across stations (Table 6). F statistic value for all the four populations is given in Table 7 and was found to be statistically significant at 1% level (Table 8). Classification matrix of four populations of *T. ilisha* from Brahmaputra River, Assam based on concentration of 14 trace elements (log transformed) in otolith shows that 100% of the total population is correctly classified for Guwahati 2018 and Dhubri 2017 populations followed by 86.67% for Guwahati 2017 and Dhubri 2018 populations (Table 9). Overall, it was seen that 93.33% of the total samples were correctly classified.

Bivariate plot shows that in general the differences in otolith micro-chemistry of *T. ilisha* are more pronounced on a spatial scale as compared to the temporal differences. The plot shows that there is a marked variation between Dhubri and Guwahati populations. The plot shows mixing between Dhubri 2017 and Dhubri 2018 population and between Guwahati 2017 and Guwahati 2018 population (Fig. 4).

## *Discussion*

Chemical archives of hard parts like otoliths, vertebrae, spines etc. can be used to interpret ecological and life-history changes[11,33]. Salmon stocks identified based on concentration of trace elements like P, Ca, K, Cu, and Sr with 80-95% accuracy. Isotopes of Sr from hard parts were used to characterize salmon stocks from different tributaries of Connecticut River[34,35]. Different researchers have also delineated stocks of wild and farmed fishes based on chemical signatures in hard parts[36,37].

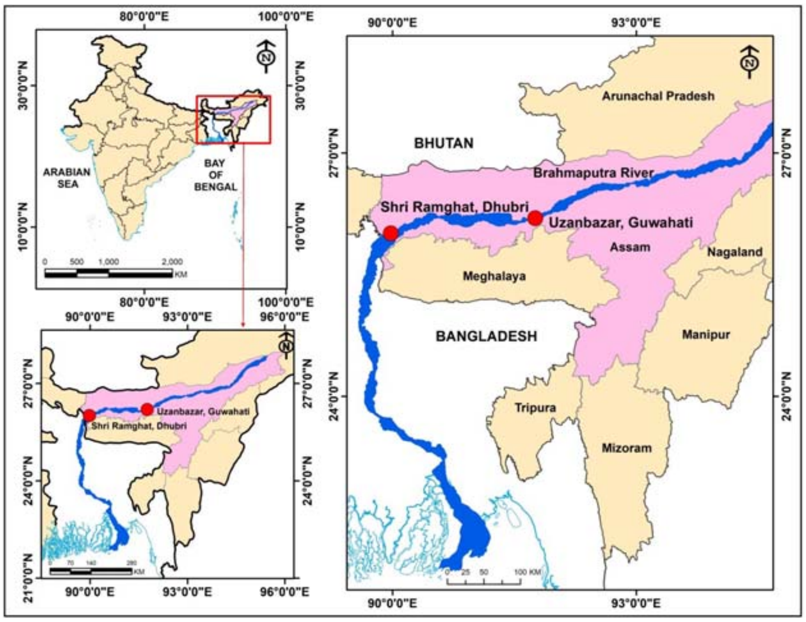


**Fig. 1:** Sampling stations in River Brahmaputra, Assam [ArcGIS Desktop: Release 9.3. (ESRI, 2008)]

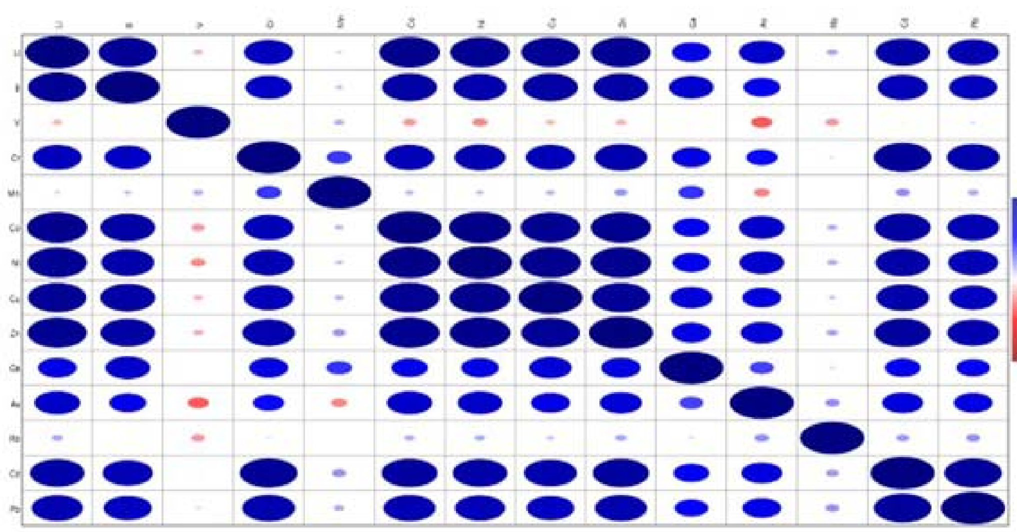

**Fig. 3:** Correlation matrix based on concentration of 14 trace elements (log transformed) in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam

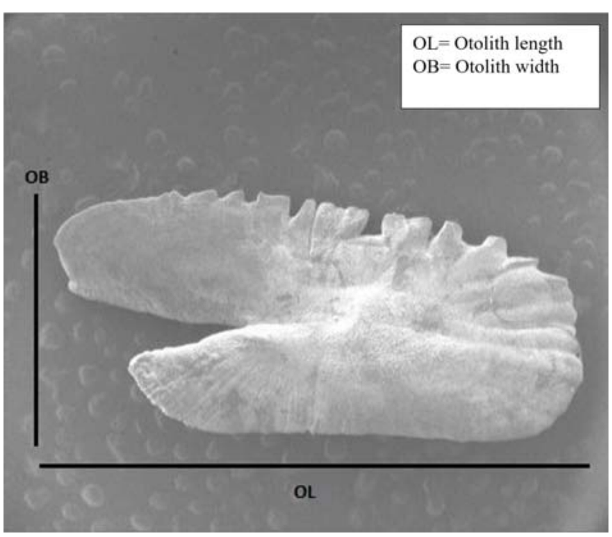


**Fig. 2:** Scanning electron microscopy image of *T. ilisha* otolith (Sagittae)

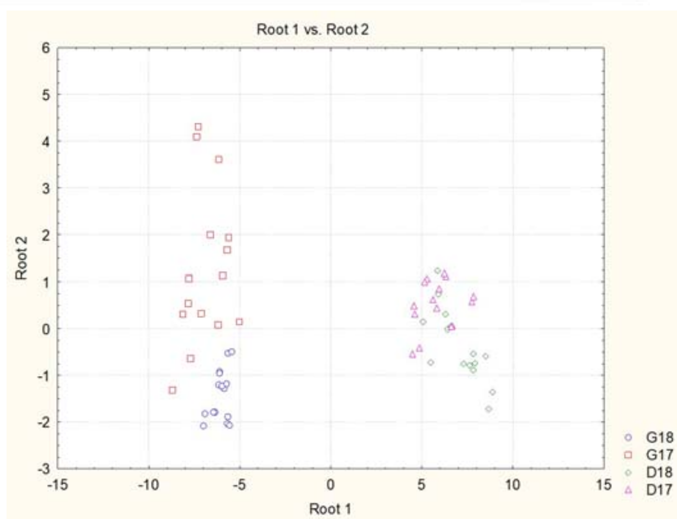


**Fig. 4:** Bivariate plot based on concentration of 14 trace elements (log transformed) in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam

**Table 2. Mean concentration (mg kg$^{-1}$) of 14 trace elements in otolith of *T. ilisha* collected from Brahmaputra River, Assam**

| Population | Guwahati 2018 | | Guwahati 2017 | | Dhubri 2018 | | Dhubri 2017 | |
|---|---|---|---|---|---|---|---|---|
| Trace element | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Li | 0.951 | 0.052 | 0.725 | 0.129 | 15.030 | 2.083 | 6.501 | 0.600 |
| Be | 0.163 | 0.036 | 0.677 | 0.401 | 1.586 | 0.201 | 0.797 | 0.061 |
| V | 4.580 | 0.455 | 4.637 | 0.561 | 3.303 | 0.218 | 4.868 | 0.478 |
| Cr | 28.975 | 1.940 | 67.200 | 20.819 | 265.099 | 28.869 | 230.548 | 29.165 |
| Mn | 20.541 | 4.787 | 27.727 | 6.242 | 16.937 | 0.835 | 26.115 | 1.122 |
| Co | 4.472 | 0.542 | 3.300 | 0.605 | 89.005 | 7.521 | 54.331 | 3.115 |
| Ni | 15.503 | 1.557 | 18.109 | 2.046 | 169.025 | 16.272 | 112.060 | 6.719 |
| Cu | 8.348 | 0.522 | 23.023 | 9.185 | 353.790 | 28.259 | 252.595 | 24.830 |
| Zn | 26.618 | 1.473 | 28.414 | 2.806 | 300.912 | 30.260 | 296.794 | 35.300 |
| Ga | 22.079 | 11.401 | 25.157 | 9.319 | 33.119 | 3.455 | 21.389 | 1.446 |
| As | 9.435 | 0.612 | 8.821 | 1.661 | 22.961 | 2.722 | 14.439 | 1.034 |
| Rb | 11.814 | 3.593 | 5.379 | 1.351 | 6.237 | 0.818 | 16.494 | 4.587 |
| Cd | 0.132 | 0.007 | 0.262 | 0.102 | 1.245 | 0.124 | 1.426 | 0.222 |
| Pb | 7.591 | 0.473 | 9.545 | 3.076 | 35.237 | 6.019 | 40.988 | 6.957 |

In the present study a total of 14 trace elements were analysed. Discriminant function analysis showed significant difference in concentration of seven trace elements in otolith of *T. ilisha* specimens collected from Dhubri and Guwahati landing centres of River Brahmaputra in Assam both on a temporal and spatial scale. However, the difference was more prominent on a spatial scale as compared to temporal scale. Trace element concentration in otoliths has been successfully used in detecting variation in fish species on a spatial scale[24-26]. Temporal variability in trace element composition in otoliths is also demonstrated by researchers over the years[25,27]. It was observed that significant variation exists in otolith trace element composition of the highly migratory Atlantic cod, *Gadus morhua*, collected from Fundyrip and Georges Bank in Atlantic Ocean[28]. Both these sites are geographically close and there is a definite mixing of Cod stocks between these two sites as observed by the authors, which support our present findings for the anadromous Hilsa.

**Table 3. Correlation matrix based on concentration of 14 trace elements (log transformed) in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam**

| Element | Li | Be | V | Cr | Mn | Co | Ni | Cu | Zn | Ga | As | Rb | Cd | Pb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Li | | 0.9127 | -0.1466 | 0.7644 | 0.0852 | 0.9444 | 0.9286 | 0.9118 | 0.9274 | 0.5993 | 0.7101 | 0.1681 | 0.8487 | 0.7999 |
| Be | 0.9127 | | -0.0395 | 0.7239 | 0.1132 | 0.8544 | 0.8273 | 0.8578 | 0.8543 | 0.6949 | 0.5726 | -0.0073 | 0.7821 | 0.7477 |
| V | -0.1466 | -0.0395 | | 0.0022 | 0.1548 | -0.2038 | -0.2317 | -0.1442 | -0.1576 | 0.0215 | -0.3224 | -0.2068 | -0.0472 | 0.0785 |
| Cr | 0.7644 | 0.7239 | 0.0022 | | 0.3906 | 0.7771 | 0.7950 | 0.7726 | 0.8198 | 0.6030 | 0.4826 | 0.0617 | 0.8988 | 0.8232 |
| Mn | 0.0852 | 0.1132 | 0.1548 | 0.3906 | | 0.1315 | 0.1207 | 0.1403 | 0.2011 | 0.3989 | -0.2417 | 0.0240 | 0.2179 | 0.1623 |
| Co | 0.9444 | 0.8544 | -0.2038 | 0.7771 | 0.1315 | | 0.9682 | 0.9295 | 0.9365 | 0.5636 | 0.7074 | 0.1545 | 0.8715 | 0.8105 |
| Ni | 0.9286 | 0.8273 | -0.2317 | 0.7950 | 0.1207 | 0.9682 | | 0.9476 | 0.9443 | 0.5839 | 0.6976 | 0.1577 | 0.8492 | 0.7823 |
| Cu | 0.9118 | 0.8578 | -0.1442 | 0.7726 | 0.1403 | 0.9295 | 0.9476 | | 0.9123 | 0.6582 | 0.6034 | 0.1113 | 0.8220 | 0.7446 |
| Zn | 0.9274 | 0.8543 | -0.1576 | 0.8198 | 0.2011 | 0.9365 | 0.9443 | 0.9123 | | 0.6106 | 0.6556 | 0.1721 | 0.8762 | 0.8110 |
| Ga | 0.5993 | 0.6949 | 0.0215 | 0.6030 | 0.3989 | 0.5636 | 0.5839 | 0.6582 | 0.6106 | | 0.3702 | -0.0842 | 0.5529 | 0.5191 |
| As | 0.7101 | 0.5726 | -0.3224 | 0.4826 | -0.2417 | 0.7074 | 0.6976 | 0.6034 | 0.6556 | 0.3702 | | 0.2196 | 0.6354 | 0.6007 |
| Rb | 0.1681 | -0.0073 | -0.2068 | 0.0617 | 0.0240 | 0.1545 | 0.1577 | 0.1113 | 0.1721 | -0.0842 | 0.2196 | | 0.1975 | 0.2144 |
| Cd | 0.8487 | 0.7821 | -0.0472 | 0.8988 | 0.2179 | 0.8715 | 0.8492 | 0.8220 | 0.8762 | 0.5529 | 0.6354 | 0.1975 | | 0.8953 |
| Pb | 0.7999 | 0.7477 | 0.0785 | 0.8232 | 0.1623 | 0.8105 | 0.7823 | 0.7446 | 0.8110 | 0.5191 | 0.6007 | 0.2144 | 0.8953 | |

*Values in bold indicate high correlation

**Table 4. Factor loadings (varimax rotation) based on principal component of concentration of 14 trace elements (log transformed) in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam**

| Variable | Factor loadings (Varimax raw) | |
|---|---|---|
| | Factor 1 | Factor 2 |
| Li | 0.959126* | -0.08937 |
| Be | 0.899251* | 0.08601 |
| V | -0.139206 | 0.66623 |
| Cr | 0.869033* | 0.26536 |
| Mn | 0.191921 | 0.72000* |
| Co | 0.963799* | -0.09933 |
| Ni | 0.959775* | -0.10856 |
| Cu | 0.940475* | -0.00485 |
| Zn | 0.962585* | -0.01523 |
| Ga | 0.679581 | 0.38942 |
| As | 0.712800* | -0.48554 |
| Rb | 0.165037 | -0.41946 |
| Cd | 0.928107* | 0.05643 |
| Pb | 0.873565* | 0.08079 |

*Values >0.70 are considered as marked loadings

**Table 5. Discriminant function analysis based on concentration (log transformed) of 10 trace elements showing significant factor loadings in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam**

| N=60 | Discriminant Function Analysis | | | | | |
|---|---|---|---|---|---|---|
| | Wilks' Lambda | Partial Lambda | F-remove | p-level | Toler | 1-Toler ($R^2$) |
| Co | 0.008433 | 0.735185 | 5.643162** | 0.002185 | 0.407816 | 0.592184 |
| Zn | 0.009194 | 0.674337 | 7.566041** | 0.000314 | 0.679477 | 0.320523 |
| Li | 0.009279 | 0.668166 | 7.780609** | 0.000255 | 0.737286 | 0.262714 |
| Ni | 0.007610 | 0.814751 | 3.562106* | 0.021018 | 0.302960 | 0.697040 |
| Cd | 0.007985 | 0.776442 | 4.510840** | 0.007331 | 0.416174 | 0.583826 |
| Cu | 0.006903 | 0.898083 | 1.777892 | 0.164272 | 0.613044 | 0.386956 |
| Pb | 0.007528 | 0.823549 | 3.356691* | 0.026518 | 0.554661 | 0.445339 |
| Cr | 0.007647 | 0.810789 | 3.656075* | 0.018907 | 0.377150 | 0.622850 |
| As | 0.007257 | 0.854351 | 2.670833 | 0.058194 | 0.526988 | 0.473012 |
| Mn | 0.007131 | 0.869425 | 2.35915 | 0.084118 | 0.514817 | 0.485138 |

**Significant at 1% level; *Significant at 5% level

**Table 6. Squared mahalanobis distance based on concentration (log transformed) of 10 trace elements showing significant factor loadings in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam**

| Population | Squared Mahalanobis Distances | | | |
|---|---|---|---|---|
| | Guwahati 2018 | Guwahati 2017 | Dhubri 2018 | Dhubri 2017 |
| Guwahati 2018 | 0.0000 | 8.4580 | 175.3692 | 146.1374 |
| Guwahati 2017 | 8.4580 | 0.0000 | 198.1405 | 165.1482 |
| Dhubri 2018 | 175.3692 | 198.1405 | 0.0000 | 6.8385 |
| Dhubri 2017 | 146.1374 | 165.1482 | 6.8385 | 0.0000 |

Analysis of the trace element composition of Hilsa otoliths from Bangladesh waters was done during 1996 and 1997[30]. Analysis showed that significant variation exists in concentration of trace elements in otoliths of Hilsa specimens collected from Chandpur and Goalando during 1996 and between Chandpur, Goalando and Rajshahi sites of Padma River during 1997. Significant variation was also noticed between year 1996 and 1997 in trace element concentration of otoliths in Hilsa specimens collected from Chandpur and Goalando[30], which is in line with our present study. All these three sites fall in the freshwater zone of the river. Trace element was observed that trace element composition in otolith of *T. toli* collected from Bangladesh waters varied across years, which he attributed to changes in water chemistry[29]. This supports our present findings, where we have seen that significant differences in trace element composition across time and space. The variation as observed in our present study may be attributed to variation in water chemistry and environment[29,30].

Water is the main source for element deposition in otoliths of fishes, followed by a small portion from food[14]. In marine fish, continuous drinking of water is the main source of trace elements in otoliths, which are assimilated via intestine, while in freshwater fish's water passing through gills is the major source of water-borne elements in otoliths[38]. Elementary

**Table 7. F statistic value based on concentration (log transformed) of 10 trace elements showing significant factor loadings in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam**

| Population | F-values, df = 4.52 | | | |
|---|---|---|---|---|
| | Guwahati 2018 | Guwahati 2017 | Dhubri 2018 | Dhubri 2017 |
| Guwahati 2018 | | 5.3240 | 110.3886 | 91.9882 |
| Guwahati 2017 | 5.3240 | | 124.7224 | 103.9549 |
| Dhubri 2018 | 110.3886 | 124.7224 | | 4.3046 |
| Dhubri 2017 | 91.9882 | 103.9549 | 4.3046 | |

**Table 8. Significance (p value) of F statistic value based on concentration (log transformed) of 10 trace elements showing significant factor loadings in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam**

| Population | p- levels | | | |
|---|---|---|---|---|
| | Guwahati 2018 | Guwahati 2017 | Dhubri 2018 | Dhubri 2017 |
| Guwahati 2018 | | 0.000033 | 0.000000 | 0.000000 |
| Guwahati 2017 | 0.000033 | | 0.000000 | 0.000000 |
| Dhubri 2018 | 0.000000 | 0.000000 | | 0.000276 |
| Dhubri 2017 | 0.000000 | 0.000000 | 0.000276 | |

profile in otolith primarily reflects the habitat in which the fish has spent a major part of its life-history[39] as compared to other habitats in which the fish might have resided or migrated through for a very short duration. Hilsa available in Brahmaputra River migrates from different regions of Bay of Bengal after spending a major part of their life in the estuarine zones adjoining Bay of Bengal region[40]. Variation in otolith micro-chemistry of Hilsa specimens collected from Dhubri and Guwahati as observed in the present study might be due to different fishes spending major part of their life-histories in different estuarine habitats with varying water chemistry and varying food availability. Element deposition in otoliths of Hilsa owing to food intake is a pure reflection of its estuarine habitat as the fish ceases to feed during the course of its upstream migration[41]. Previous research has also indicated significant differences in trace elemental composition in otoliths of Hilsa as well as in water samples collected from different coastal regions of Bangladesh[30].

There is a possibility that variation in sex, age, length of fish and weight of otolith may play a role in the variation of trace element concentration. However, work of previous researchers indicated that sex and otolith weight[30], sex and age[29] or age, sex and length of fish[28] do not play any significant role in variation of trace element concentration in otoliths. During the present study, Hilsa specimens were collected from Dhubri and Guwahati landing centers of River Brahmaputra, Assam at the time of their spawning (October), which was also evident from the mature/ ripe individuals sampled in the period. Hilsa migrates into Brahmaputra for spawning and numerous spawning grounds of the species were located along the river[42] Spawning aggregation of the species might also result in distinct populations[43] and otolith trace element concentration is bound to vary across populations. However, in comparison to the theory of distinct population, water chemistry might have played a more significant role in this regard.

Relevant information was generated in the present study on trace elemental composition in otoliths of *T. ilisha* from Brahmaputra River, India. There lies enough scope for researchers working in this field to generate data on otolith trace element composition of the species from Brahmaputra River in Bangladesh and from estuarine zones adjoining Bay of Bengal region in near future. Knowledge on otolith microchemistry supplemented with relevant additional information can help researchers and managers in identifying the migratory routes, places of origin etc. of the species, which can form the basis towards sustainable management of the species on a joint venture mode involving India and Bangladesh.

**Table 9. Classification matrix of four populations of *T. ilisha* from Brahmaputra River, Assam based on concentration (log transformed) of 10 trace elements showing significant factor loadings in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam**

| Population | % correct | Guwahati 2018 (p=0.25) | Guwahati 2017 (p=0.25) | Dhubri 2018 (p=0.25) | Dhubri 2017 (p=0.25) |
|---|---|---|---|---|---|
| Guwahati 2018 | 100.0000 | 15 | 0 | 0 | 0 |
| Guwahati 2017 | 86.6667 | 2 | 13 | 0 | 0 |
| Dhubri 2018 | 86.6667 | 0 | 0 | 13 | 2 |
| Dhubri 2017 | 100.0000 | 0 | 0 | 0 | 15 |
| **Total** | **93.3333** | **17** | **13** | **13** | **17** |

Rows= Observed classifications; Columns= Predicted classifications

**Table 10. Summary of stepwise discriminant analysis based on concentration (log transformed) of 10 trace elements showing significant factor loadings in otolith of four populations of *T. ilisha* from Brahmaputra River, Assam**

| Trace element | Step | F value | df 1 | df 2 | P value | Number of variables | Lambda | F value | df 1 | df 2 | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Co | 1 | 254.4335 | 3 | 56 | 0.000000 | 1.00000 | 0.068351 | 254.4335 | 3 | 56 | |
| Zn | 2 | 13.6635 | 3 | 55 | 0.000001 | 2.00000 | 0.39163 | 74.3074 | 6 | 110 | 0.00 |
| Li | 3 | 9.3827 | 3 | 54 | 0.000043 | 3.00000 | 0.025744 | 51.1407 | 9 | 132 | 0.00 |
| Ni | 4 | 7.6566 | 3 | 53 | 0.000242 | 4.00000 | 0.017960 | 41.7903 | 12 | 141 | 0.00 |
| Cd | 5 | 7.2964 | 3 | 52 | 0.000357 | 5.00000 | 0.012640 | 37.1510 | 15 | 144 | 0.00 |
| Cu | 6 | 3.7711 | 3 | 51 | 0.016066 | 6.00000 | 0.010345 | 32.4356 | 18 | 145 | 0.00 |
| Pb | 7 | 2.1990 | 3 | 50 | 0.099732 | 7.00000 | 0.009139 | 28.3450 | 21 | 144 | 0.00 |
| Cr | 8 | 2.2902 | 3 | 49 | 0.089896 | 8.00000 | 0.008015 | 25.4568 | 24 | 143 | 0.00 |
| As | 9 | 1.9837 | 3 | 48 | 0.128963 | 9.00000 | 0.007131 | 23.1251 | 27 | 141 | 0.00 |
| Mn | 10 | 2.3529 | 3 | 47 | 0.084118 | 10.00000 | 0.006200 | 21.1251 | 30 | 139 | 0.00 |


## Acknowledgements

Authors are grateful to the Director, ICAR-CIFE, Mumbai for providing necessary facilities to carry out the research work. The first author thankful to the Director, ICAR-CIFRI, Barrackpore, Kolkata for providing necessary laboratory facilities and valuable guidance for completing the research work. The authors also thankful to the fisher community of river Brahmaputra, Assam, India. ❐


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