



## Association pattern between dimensions of fish and otolith to expedite morphometric variations of three geographically isolated stocks of *Tenualosa ilisha* (Hamilton, 1822) from diverse ecosystems

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### ABSTRACT

An attempt was made to establish association pattern between dimension of fish and otolith to expedite morphometric variations of geographically isolated stocks of *Tenualosa ilisha* (Hamilton, 1822), from three diverse ecosystems; viz., an estuarine (brackish water), a riverine (freshwater) and a lacustrine (freshwater) ecosystem. Sampling was carried out during the period from September 2017 to February 2019 and a total of 196 otoliths were sampled from Narmada Estuary (n=69), Brahmaputra River (n=75) and Ukai Reservoir (n=52) of India. Mean otolith length recorded was 3.55±0.14 mm, 5.18±0.09 mm and 3.85±0.07 mm for samples collected from Narmada Estuary, Brahmaputra River and Ukai Reservoir respectively. Otolith length-weight relationship (OLWR) from three ecosystems showed negative allometric *b* values ranging from 1.9046-2.5048, with significant difference between Narmada Estuary and Brahmaputra River ( $p < 0.05$ ). Power regression analysis of otolith dimensions (length and width) to total length of fish (TL) and otolith length (OL) to total weight of fish (TW) showed that data fitted well to the model as evident from  $R^2$  values ( $> 0.6$ ); except for relationship between otolith width (OB) and TL in samples from Brahmaputra River ( $R^2 = 0.459$ ). Present study provides first-hand information on association pattern of dimension of fish body and otolith of *T. ilisha* which can be used in geographical mapping of stock profile of the species.

Keywords: Estuarine, Hilsa, Lacustrine, Otolith, Riverine, *Tenualosa ilisha*

### Introduction

Otolith has been used for estimating size of fish (Templemann and Squires, 1956; Echeveria, 1987) which is accomplished by formulating otolith dimensions and fish length relationship (Echeveria, 1987; Gamboa, 1991; Waessle *et al.*, 2003; Tarkan *et al.*, 2007). Besides, information available from annual rings of otolith is an important tool in stock assessment studies to assess fish age and growth as well as in studying feeding habits (Harvey *et al.*, 2000). Otoliths also have use in paleontological studies (Nolf, 1995) and are also used in studies related to population and community ecology (Feyrer *et al.*, 2007). Microchemistry analyses of otoliths has enabled fishery scientists to study pattern of fish migration (Kennedy *et al.*, 2002), preferred habitats at different life-history stages (Kennedy *et al.*, 1997; Hobbs *et al.*, 2005) as well as in stock identification (Campana *et al.*, 2000). Otoliths, particularly sagittae are primarily used in studies related

to age determination (Tracey and Horn, 1999; Vieira *et al.*, 2013). Besides it is also used in taxonomical studies owing to its taxonomical distinctness (Longenecker, 2008) and species-specificity (Morrow, 1979) and in studies on trophic level (Campana *et al.*, 2000; Sadighzadeh *et al.*, 2014) by determining prey composition (Pierce and Boyle, 1991; Battaglia *et al.*, 2010).

*Tenualosa ilisha* (Hamilton, 1822) commonly known as hilsa is an anadromous fish reported from varied riverine and estuarine ecosystems in India, ranging from the snowfed Himalayan rivers like Ganga, Bhagirathi, Hooghly, Rupnarayan and Brahmaputra and their estuaries to rainfed peninsular rivers like Godavari and their estuaries, all of which drains into Bay of Bengal. The species is also reported from rainfed peninsular rivers like Narmada and Tapti and their estuaries (Bhaumik and Sharma, 2012) draining into Arabian Sea. Rivers Hooghly, Ganga, and Brahmaputra along with their tributaries contribute a significant portion of about

70% of the total hilsa production in India (Raja, 1985). The species has a great cultural and economic importance in India and Bangladesh. It has a very high consumer preference, highly prized (Sahoo *et al.*, 2016) and is designated as the national fish of Bangladesh. Mohanty *et al.* (2012) reported that this species is rich in omega-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The importance of this species is evident from the assessment made by Bay of Bengal Large Marine Ecosystem (BOBLME) project. The economic value of hilsa fishery is worth over US\$ 2 billion and generates employment opportunities as well as acts as livelihood source for millions of people in India, Bangladesh and Myanmar (BOBLME, 2012). Existence of separate hilsa stocks in Indian waters has been confirmed through cytochrome *b* gene analysis, which showed that hilsa of Bay of Bengal origin is completely distinct from Arabian Sea (Behera *et al.*, 2015). Hilsa found in Narmada Estuary and Ukai Reservoir are of Arabian Sea origin (Bhaumik, 2013), while hilsa in Brahmaputra are of Bay of Bengal origin (Miah, 2015). The present study provides information on comparison of existing relationship between otolith dimensions *viz.*, otolith length (OL) and otolith width (OB) with total length (TL) and total body weight (TW) of fish along with information on otolith length-weight relationship (OLWR) and length-weight relationship (LWR) of fish in three geographically isolated populations of *T. ilisha* collected from three different ecosystem types *viz.*, brackishwater and estuarine (Narmada Estuary), freshwater and riverine (Brahmaputra River) and freshwater and lacustrine (Ukai Reservoir on

Tapti river) of India.

## Materials and methods

### *Sample collection, preservation and identification*

Samples were collected from five different locations comprising estuarine, riverine and lacustrine habitats (Fig. 1, Table 1) during September 2017 to February 2019. Specimens were obtained from commercial catches, caught using drift gillnets and bag nets of mesh size 40-80 mm and 10-15 mm respectively. Specimens caught fresh were preserved in 10% neutral buffered formalin and brought to the laboratory for analysis. Specimens were identified following standard manuals (Talwar and Jhingran, 1991).

### *Length-weight relationship (LWR)*

Prior to extraction of otolith, length and weight of 196 specimens were recorded from Narmada Estuary (n=69), Brahmaputra River (n=75) and Ukai Reservoir (n=52). Total weight (TW) and total length (TL) of the specimens were measured with the help of a scale (0.1 cm accuracy) and electronic precision balance (0.01g accuracy) respectively. Outliers were removed before subjecting the data for establishing LWR of the species, using log W vs log TL plots (Froese, 2006). Using MS Excel 2010, total length and total weight data of collected hilsa specimens were subjected to log transformation individually, before carrying out linear regression analysis using the equation:  $\log W = \log a + b \log TL$  (Le Cren, 1951). Goodness of fit was measured by coefficient of determination ( $R^2$ ).

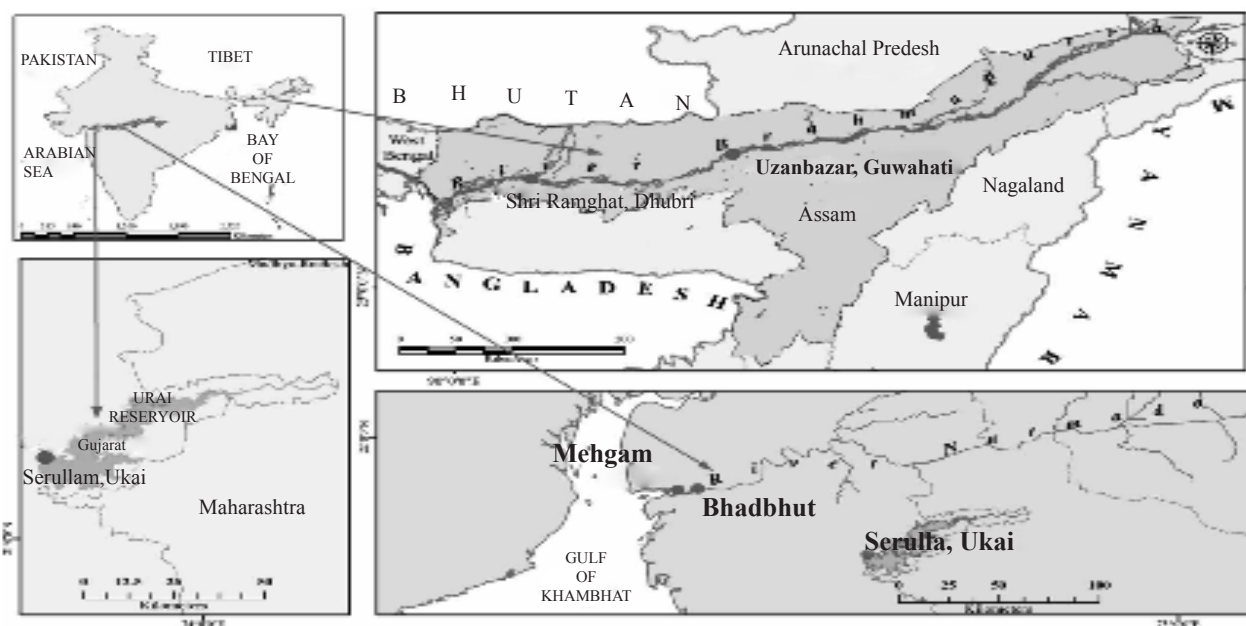


Fig. 1. Schematic map showing sampling locations in Brahmaputra River, Narmada Estuary and Ukai Reservoir

Table 1. Description of sampling locations, sampling period and sample size of *T. ilisha*

Ecosystem	Sampling stations	Coordinates	Sampling period	Sample size (n)
Narmada Estuary	Mehgam	21°40'26" N; 72°45'23" E	July 2018 - February 2019	69
	Bhadbhut	21°40'52" N; 72°50'42" E		
Brahmaputra River	Shri Ramghat, Dhubri	26°0'36" N; 89°58'41" E	September - December 2017; May to December 2018	75
	Uzanbazar, Guwahati	26°11'44" N; 91°45'24" E		
Ukai Reservoir	Serulla	21°16'32" N; 73°36'24" E	January - February 2018; June 2018; January - February 2019	52

#### Otolith removal and measurement

After recording length and weight data of each fish specimen, otoliths were extracted from samples collected from Narmada Estuary (n=69), Brahmaputra River (n=75) and Ukai Reservoir (n=52). Otoliths (sagittae) were taken out by making an incision around the skull (cranium). Otoliths thus collected were cleaned with distilled water and air-dried, prior to storage in vials. Otolith length (OL) and otolith breadth (OB) were measured in millimetres with a digital vernier calliper (0.01 mm accuracy). OL refers to the longest distance between frontal and hindmost points while OB refers to the maximum distance between dorsal and ventral border through its focus and measured at right angles to OL (Fig. 2). Weight of individual otolith (OW) was taken in milligrams using an electronic precision balance having 0.01 g accuracy.

#### Relationship between dimensions of fish and otolith

Power regression model was used to establish relationship between otolith dimensions (OL and OB) and TL and TW following Le Cren (1951) and Zar (1984) and using the equation:  $Y = a * X^b$ .

#### Otolith length-weight relationship (OLWR)

OLWR was established using linear regression analysis ( $\log W = a + b \log L$ ) using MS Excel, 2010. Goodness of fit was measured by coefficient of determination ( $R^2$ ).

#### Statistical analysis

Paired t - test to measure the significant differences between left and right otolith measurements (n = 30) and one way analysis of variance (Post-hoc Turkey test) to estimate the significant differences in regression co-efficient 'b' in LWR of fish and OLWR was carried out using SPSS 16.0 (SPSS, 2008).

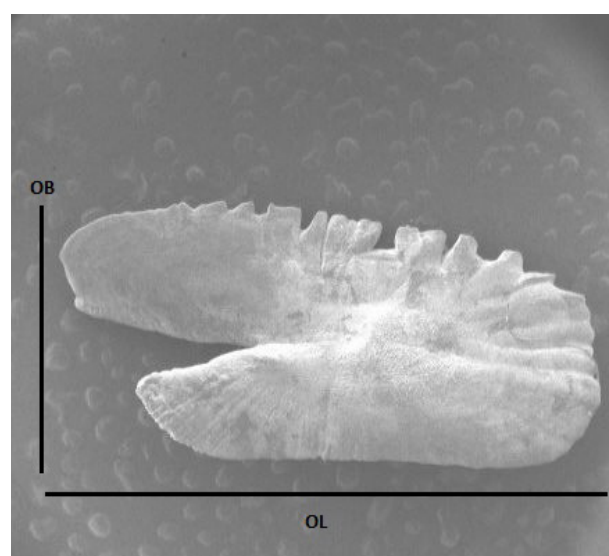


Fig. 2. Scanning electron microscopy image of *T. ilisha* otolith (Sagittae OL: otolith length, OB: Olotith breadth)

#### Results

Estimation of LWR of fish showed *b* value of 2.95, 3.02 and 3.10 for samples collected from Brahmaputra River, Narmada Estuary and Ukai Reservoir respectively.  $R^2$  value ranged from 0.92-0.99 and higher  $R^2$  value indicates higher degree of correlation in LWRs of the species studied from all three ecosystems (Table 2). One way ANOVA revealed that there is no significant difference in *b* value derived from LWR between Narmada Estuary and Ukai Reservoir ( $p > 0.05$ ), while significant difference was observed between Brahmaputra River and Narmada Estuary and between Brahmaputra River and Ukai Reservoir ( $p < 0.05$ ).

With regard to otolith, paired t - test didn't show any significant difference between left and right otolith measurements ( $p > 0.05$ ), hence left otolith was used in our study. The details of basic statistics for size and weight measurements of specimens and otoliths are given

Table 2. Estimated LWR parameters of *T. ilisha* collected from three different ecosystems

Ecosystem	n	Length-weight relationship				
		a <sup>#</sup>	95% CI of a	b	95% CI of b	R <sup>2</sup>
Narmada Estuary	69	0.009	0.008-0.011	3.02 <sup>c</sup>	2.99-3.05	0.99*
Brahmaputra River	75	0.010	0.008-0.013	2.95 <sup>d</sup>	2.81-3.10	0.97*
Ukai Reservoir	52	0.006	0.004-0.008	3.10 <sup>c</sup>	3.02-3.19	0.92*

a and b: regression parameters; CL: confidence limit; R<sup>2</sup>: co-efficient of determination; <sup>#</sup>Antilog a; \*p<0.01  
Values with different superscript indicate significant difference (p<0.05)

in Table 3. Mean OL (3.55±0.14 mm) was found to be lowest in samples collected from brackishwater estuarine ecosystem (Narmada Estuary) and highest (5.18±0.09mm) in freshwater riverine ecosystem (Brahmaputra River) with intermediate values (3.85±0.07 mm) in freshwater lacustrine ecosystem (Ukai Reservoir). Power regression analysis of OL to TL and TW showed that data fitted well to the model in samples from all three ecosystems (R<sup>2</sup>>0.6) with a greater degree of fitting in Narmada Estuary (R<sup>2</sup>>0.9).

Higher correlation was observed in relationship between OB and TL in case of samples from Narmada Estuary (R<sup>2</sup>=0.95) and Ukai Reservoir (R<sup>2</sup>=0.84) but moderate R<sup>2</sup> value was obtained for Brahmaputra River (R<sup>2</sup>=0.46) (Table 4). OLWR from these three ecosystems showed negative allometric b values ranging from 1.9046-2.5048, in the order of freshwater lacustrine<brackishwater estuarine<freshwater riverine ecosystems (Table 5). One way ANOVA showed significant difference in b value derived from OLWR between Narmada Estuary and Brahmaputra River (p<0.05), but did not show significant

difference (p>0.05) between Brahmaputra River and Ukai Reservoir (p=0.07) and between Narmada Estuary and Ukai Reservoir (p=0.72).

### Discussion

A number of studies on LWR of hilsa have been reported from Indian waters (Mandal *et al.*, 2018; Bhakta *et al.*, 2019). Mandal *et al.* (2018) reported higher b value for *T. ilisha* collected from Ukai Reservoir compared to Narmada and east-flowing rivers like Padma which is in line with our findings. Significant variation (p<0.05) in growth pattern of hilsa from Brahmaputra River as compared to Ukai Reservoir and Narmada Estuary may be attributed to geographical and environmental discreteness along with notable differences in environmental factors *viz.*, temperature and food availability (Turan, 2004). Genetic differentiation might also have played a key role in this regard. High degree of genetic variation exists in hilsa populations of Bay and Bengal and Arabian sea region (Behera *et al.*, 2015); between Brahmaputra and Narmada (NBFGR, 2016), while low percentage of genetic variation

Table 3. Basic statistics for fish and otolith size and weight measurements Mean±SE of *T. ilisha* collected from three different ecosystems

Ecosystem	Ecosystem type	n	Total length (cm)	Total weight (g)	Otolith length (mm)	Otolith weight (mg)	Otolith width (mm)
Narmada Estuary	Brackishwater; Estuarine	69	17.75±1.03	62.95±8.79	3.55±0.14	2.90±0.23	1.59±0.06
Brahmaputra River	Freshwater; Riverine	75	28.11±0.71	215.90±16.97	5.18±0.09	7.67±0.37	2.30±0.04
Ukai Reservoir	Freshwater; Lacustrine	52	16.31±0.36	40.94±3.12	3.85±0.07	4.27±0.17	1.78±0.03

n: Sample size; SE: Standard error

Table 4. Power regression relationships between fish size and weight measurements and otolith measurements of *T. ilisha*

Ecosystem	Exponential formula	R <sup>2</sup>
Narmada estuary	TL = 2.849 OL <sup>1.425</sup>	0.929
	TL = 8.391 OB <sup>1.525</sup>	0.945
	TW = 0.287 OL <sup>3.981</sup>	0.930
Brahmaputra river	TL = 3.432 OL <sup>1.274</sup>	0.723
	TL = 11.395 OB <sup>1.074</sup>	0.459
	TW = 0.303 OL <sup>3.934</sup>	0.674
Ukai reservoir	TL = 4.690 OL <sup>0.921</sup>	0.642
	TL = 7.998 OB <sup>1.231</sup>	0.840
	TW = 0.780 OL <sup>2.889</sup>	0.610

R<sup>2</sup>:co-efficient of determination

Table 5. Relationship between otolith length and otolith weight of *T. ilisha* collected from three different ecosystems

Ecosystem	type Ecosystem	n	Relationship between	Regression parameters for OLWRs				Standard error (SE) of regression parameters		Correlation coefficient
				$a^{\#}$	95% CL of $a$	$b^*$	95% CL of $b$	SE ( $a$ )	SE ( $b$ )	
Narmada Estuary	Brackishwater; Estuarine	69	OL X OW	0.2193	0.1744-0.2759	1.9868 <sup>c</sup>	1.8051-2.1685	0.0492	0.0897	0.9299
Brahmaputra River	Freshwater; Riverine	75	OL X OW	0.1208	0.0515-0.2833	2.5048 <sup>d</sup>	1.9861-3.0235	0.1822	0.2552	0.7391
Ukai Reservoir	Freshwater; Lacustrine	52	OL X OW	0.3222	0.1898-0.5468	1.9046 <sup>cd</sup>	1.5122-2.2969	0.1123	0.1918	0.7727

$a$  and  $b$ : regression parameters; CL, confidence limit;  $R^2$ , co-efficient of determination;  $\#$ Antilog  $a$ ;  $*p < 0.01$   
Values with different superscript indicate significant difference ( $p < 0.05$ )

was is reported between hilsa population from Ukai and Narmada (NBFG, 2017). Previous bibliographic studies revealed relationship between fish size and otolith dimensions (Metin and Ilkyaz, 2008; Jawad *et al.*, 2011). In the present study, impression of somatic growth exerting significant influence on otolith growth can be derived from correlation existing between fish size and otolith size (Munk, 2012). Positive correlation existing between OL and OB with TL has been reported in *Sardinella sindensis* (Dehghani *et al.*, 2016) and in *Amblypharyngodon mola* (Nimesh and Jain, 2018). In majority of the species, a simple linear regression best describes the relationship between TL and OL (Battaglia *et al.*, 2010; Battaglia *et al.*, 2015). On the contrary, Leonart *et al.* (2000) stated, linear model is unsuitable in studying relationships between OL and TL, as such models are unable to detect changes in shape and parameter ' $a$ ' do not hold much importance in morphometrics, hence a power regression model is the best fit. Kumar *et al.* (2016) found significant positive correlation between TL and TW with OL and OB in four marine fishes from Indian waters using a power regression model, which is in accordance to present study, except for the relationship between TL and OB from Brahmaputra River, which showed comparatively lower  $R^2$  value.

Significant differences in dimensions were not observed for left and right otoliths or for otoliths of both sexes in clupeid, *S. sindensis* (Dehghani *et al.*, 2016) and between left and right otolith in *A. mola* (Nimesh and Jain, 2018), which supported our present study. Variation in place of origin, leading to variation in stocks might play a significant role in dissimilarities in OLWR. Intra-specific variation in otolith shape and appearance are recorded in separate stocks (Campana and Casselman, 1993; Begg *et al.*, 2001; Cardinale *et al.*, 2004). In the present study, significant differences are observed in OLWR between samples of Narmada Estuary and Brahmaputra River, while, no such difference was noted between Narmada Estuary and Ukai Reservoir.

Very limited studies have been made on otolith morphology and relationship between fish TL and TW with otolith dimensions in Indian marine fishes. Such studies are more limited in case of freshwater and diadromous fishes of India. The present attempt to study OLWR and relationship existing between otolith dimensions (OL and OB) with TW and TL of *T. ilisha* from brackishwater estuarine, freshwater riverine and freshwater lacustrine ecosystems, is one of the first of its kind from Indian waters.

During our study it was found that size and weight of otoliths increase with increase in size and weight of fish, thereby, confirming a positive correlation between fish size and otolith size. Study on OLWR of *T. ilisha* revealed significant variation between Brahmaputra River and Narmada Estuary. Thus, the present study will provide comparative information to researchers on the pattern of relationship existing between otolith size and fish size across different types of ecosystems.

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