10.15517/RBT.V67I3.33985
Artículo

Using otolith weight to predict the age of different stocks of *Sperata aor* (Siluriformes: Bagridae) from the River Ganga

Peso de otolitos para predecir la edad de diferentes cohortes de *Sperata aor* (Siluriformes: Bagridae) en el Río Ganga

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Received 28-XI-2018
Corrected 10-IV-2019
Accepted 30-IV-2019

Abstract
Estimation of accurate fish age is considered as an essential step for the understanding of life history characteristics, population dynamics, and the management of the fisheries resources. The otolith weight keeps on increasing because of continuous deposition of material on the otolith surface; therefore, otolith measurements are successfully used to infer fish age. The present study was conducted to estimate the relationship between otolith weight and observed age (estimated by counting annuli on the sectioned otoliths) for the stocks of *Sperata aor*. A total of 315 samples were collected from January 2016 to April 2017 from three different stocks of *S. aor* i.e. Narora-Kanpur, Varanasi and Bhagalpur from the River Ganga. Linear regression analysis was applied between otolith weight and observed age to predict the age of the fish of each stock from otolith weight. Significant relationships between otolith weight and fish age were observed for the three stocks of the selected fish species from the River Ganga ($R^2 > 0.9$, $P < 0.001$). No significant differences were found in the otolith weight among the stocks of *S. aor* (ANCOVA: $F_{2,134} = 0.047$; $P > 0.05$). Overall, 88.5, 88.8, and 87.2 % of the predicted ages were correctly classified to their observed ages for Narora-Kanpur, Varanasi, and Bhagalpur stock, respectively. Thus, it can be concluded that the relationship between otolith weight and fish age can provide a surrogate method of age estimation, and can be used to examine the age structure of the three stocks of *S. aor* from the River Ganga.
Key words: age prediction; otolith weight; stocks; long-whiskered catfish; River Ganga.

Resumen
La estimación precisa de la edad de peces es considerada un paso esencial para la evaluación de su historia natural, dinámica de población y manejo de pesquerías. El otolito sigue creciendo debido a la continua deposición de material en la superficie; por lo tanto, las medidas del otolito son un buen indicador para inferir la edad del pez. En este estudio se evaluó la relación entre el peso de los otolitos y la edad observada (estimada contando los anillos de los otolitos seleccionados) de individuos de *Sperata aor.* En total se recolectaron 315 muestras entre enero 2016 y abril 2017 en tres zonas de *S. aor* en el Río Ganga (Narora-Kanpur, Varanasi y

Bhagalpur). Se aplicó un análisis de regresión lineal entre el peso de los otolitos y la edad observada para predecir la edad de los peces de cada zona a partir del peso de los otolitos. Se observaron relaciones significativas entre el peso de los otolitos y la edad de los peces de las zonas del Río Ganga ($R^2 > 0.9$, $P < 0.001$). También se encontraron diferencias significativas en el peso de los otolitos de las diferentes clases de edad (ANOVA: $F_{2,114} = 3914.03$; $P < 0.001$) mientras que no fue así entre el peso de los otolitos y las distintas zonas de recolecta de *S. aor* (ANCOVA: $F_{2,147} = 0.043$; $P > 0.05$). En general, 88.5, 88.8 y 87.2% de las edades predichas se clasificaron correctamente con respecto a las edades observadas para Narora-Kanpur, Varanasi y Bhagalpur, respectivamente. Se puede concluir que la relación entre el peso de los otolitos y la edad de los peces puede proveer un método para la estimación de la edad y puede ser usado para examinar la estructura de edades en tres *stocks* de *S. aor* en el Río Ganga.
Palabras clave: predicción de edad; peso de otolitos; bagre; Río Ganga.

Introduction

Age and growth estimation of fish populations is one of the important aspects in stock assessment, population dynamics and successful fisheries management (Pino, Cubillos, Araya, & Sepulveda, 2004; Yan, Wu, Lu, Li, & Jin, 2009). Traditionally, fish age is estimated by counting the annuli in calcified structures (Yan et al., 2009). However, the preparation process is normally time-consuming, expensive and requires expertise (Cardinale, Arrhenius, & Johnsson, 2000). Fish age could be better estimated by otolith weight than other otolith measurements such as otolith length, width and thickness, and even fish length (Boehlert, 1985; Newman, 2002; Francis & Campana, 2004). Several studies have presented the evidence of a close relationship between otolith weight and age for both tropical and temperate fishes (Fletcher, 1991; Worthington, Doherty, & Fowler, 1995; Cardinale & Arrhenius, 2004; Lou, Mapstone, Russ, Davies, & Begg, 2005; Yan et al., 2009; Britton & Blackburn, 2014; Khan, Nazir, & Banday, 2018). The otolith weight increases continuously during the life of fish in contrast to otolith length and fish length (Fowler & Doherty, 1992). Therefore, the otolith weight can be used as an effective substitute for age to reduce the cost and subjectivity associated with traditional annuli enumeration (Francis & Campana, 2004; Britton & Blackburn, 2014). It is reported that otolith weight could be used to estimate age structures of fish populations besides estimating ages of individual fish (Worthington et al., 1995; Cardinale et al., 2000). Two main factors were considered to have the potential to undermine the use of otolith weight to estimate age. The first factor is the overlap in the range of otolith weights between fish of different ages and this would decrease the ability of otolith weight to discriminate between different age-classes and could affect the frequency and magnitude of random errors in age determination. It can be overcome to some degree by recurrent recalibrating of the relationship between otolith weight and age (Worthington et al., 1995), it is likely to be a product of natural variation in growth of the fish and their otoliths and so a constant source of uncertainty in ages predicted from otolith weights (Lou et al., 2005). The second factor is the spatial or temporal variation in the relationship between otolith weight and age, for example, spatial variation in the slope of the relationship could bias subsequent age determinations if the relationship from one geographic area were applied in another (Worthington et al., 1995; Lou et al., 2005). The second factor has not been investigated well in published literature and warrants more thorough assessment over wide spatial and temporal scales (Lou et al., 2005).

Long-whiskered catfish, *Sperata aor* (Hamilton, 1822), is a commercially important freshwater catfish with a strong consumer preference mainly due to its good taste and fewer number of intramuscular bones (Talwar & Jhingran, 1991). *Sperata aor* is distributed in the rivers, ponds, lakes, tanks, channels and reservoirs of India, Pakistan, Bangladesh, Nepal and upper Myanmar (Talwar & Jhingran, 1991). This fish species is extremely sensitive to water quality variations and this may be the reason of discernible shift in its distribution pattern. Earlier, the fish was mainly caught from the middle and lower regions of the River Ganga but now it is also reported from the upper region as well (Menon, 1954; Sarkar et al., 2012). In India, *S. aor* has been considered vulnerable due to its habitat conditions and anthropogenic threats such as, dense human settlements along the rivers, damming and water diversions, release of untreated wastes, destruction of breeding grounds by sedimentation and climate change (Lakra, Sarkar, Gopalakrishnan, & Kathirvelpandian, 2010; Sarkar et al., 2012; Nazir & Khan, 2017). Several studies reported the presence of three stocks (Narora-Kanpur, Varanasi and Bhagalpur) of *S. aor* in the River Ganga using microsatellite markers and truss morphometry of the fish body (Nazir & Khan, 2017; Khan & Nazir, 2018).

A better understanding of basic biological parameters is very essential for proper management of the *S. aor* stocks in the River Ganga. Therefore, this study was conducted to evaluate the relationship between otolith weight and observed age (calculated by reading annuli on sectioned otoliths) in the stocks of *S. aor* from the River Ganga.

Materials and methods

Sampling: In total, 315 samples were collected by using seine nets and gill nets from January 2016 to April 2017 from three different stocks of *S. aor* i.e. Narora (27˚30' N & 78˚25' E)-Kanpur (26˚28' N & 80˚24' E), Varanasi (25˚30' N & 83˚01' E) and Bhagalpur (25˚16' N & 87˚01' E) from the River Ganga. The collected fish samples were identified according to Talwar and Jhingran (1991). The samples were transported in the ice box to the laboratory where they were measured (fish length, cm), their otoliths removed, cleaned and washed with distilled water. Otoliths were then dried at room temperature for 24 h before being weighed (nearest mg).

Preparation of otoliths for thin-sectioning and age reading: Otoliths were prepared for thin sectioning (approximately 0.5 mm) by using IsoMet® Low Speed Saw (Buehler Lake Bluff, United States) according to the protocol detailed in Khan, Nazir, and Khan (2016). The thin-sections were polished using 1200-grit CarbiMet® 2 Abrasive Discs and subsequently examined under Nikon® SMZ745T stereozoom microscope for age estimation.

Data analysis: To predict the age of the fish of each stock from otolith weight, linear regression analysis was applied between otolith weight and observed age (age obtained from counting annuli on the sectioned otoliths), that is,

$$T = \frac{OW - a}{b}$$

where *T* is the estimated age (years), OW the otolith weight, *a* is the intercept and *b* is the slope of linear regression between OW and observed age, respectively (Cardinale et al., 2000). The relationship between otolith weight and age was compared among stocks of the selected fish species using analysis of covariance (ANCOVA).

In the discriminant function analysis, we used observed age (estimated by counting annuli on sectioned otoliths) of each specimen as a grouping variable and its otolith weight and total fish length as independent variables. For each discriminant function, the predicted ages and their associated probability were saved, thus the analysis exhibited the proportion of fish classified by age according to their relationship between otolith weight and fish length vs their observed age obtained by counting annuli on the sectioned otoliths (Britton & Blackburn, 2014). The contribution of the predicted ages to the observed ages were shown by the scatterplots for the three stocks of *S. aor*. All statistical tests were performed using MS-Excel (Microsoft Corporation, Redmond, WA, USA) and SPSS version 23.0.

Results

The number of fish samples and mean otolith weight at each age for the three stocks of *S. aor* are presented in table 1 (Table 1). A significant relationship between fish total length and otolith weight was observed ($R^2 > 0.9$, $P < 0.001$) for the three stocks (Table 2). Significant relationships between otolith weight and fish age were observed for the stocks of the selected fish species from the river Ganga ($R^2 > 0.9$, $P < 0.001$) (Fig. 1). No significant differences were found in the otolith weight among the stocks of *S. aor* (ANCOVA: $F_{2,134} = 0.047$; $P > 0.05$). The discriminant function analysis for *S. aor* stocks revealed the first canonical discriminant function elucidating 98.8 %, of the variance in the three stocks i.e. Narora-Kanpur, Varanasi and Bhagalpur and effectively separated the fish by age groups (Wilks' $\lambda \geq 0.003$; $P < 0.001$, in all the three stocks).

TABLE 1

Number of samples (n), mean fish total length (TL) in centimeters and mean otolith weight (OW) in milligrams at each age for three stocks of *Sperata aor* form River Ganga

Age	**Narora-Kanpur**			**Varanasi**			**Bhagalpur**		
	N	TL	OW	n	TL	OW	N	TL	OW
1	19	25.8 ± 0.6	7.6 ± 0.4	16	26.1 ± 0.8	7.8 ± 0.3	18	25.9 ± 0.6	7.7 ± 0.3
2	16	37.2 ± 1.1	17.3 ± 0.9	15	36.9 ± 1.1	17.6 ± 0.9	13	37.8 ± 1.2	17.4 ± 0.8
3	15	46.8 ± 0.7	23.8 ± 0.3	16	46.5 ± 0.7	23.8 ± 0.3	15	46.1 ± 0.9	24.1 ± 0.4
4	12	54.6 ± 1.1	37.3 ± 1.2	11	55.8 ± 1.1	36.3 ± 1.1	12	56.1 ± 1.1	36.1 ± 1.1
5	10	61.2 ± 0.7	43.6 ± 0.4	10	61.9 ± 0.7	43.5 ± 0.5	11	62.2 ± 0.7	42.7 ± 0.6
6	9	67.8 ± 1.5	48.4 ± 1.1	10	68.4 ± 1.5	48.9 ± 1.0	9	67.8 ± 1.5	49.1 ± 1.0
7	9	72.5 ± 0.4	53.7 ± 0.6	10	72.9 ± 0.6	54.7 ± 0.6	10	72.5 ± 0.4	52.7 ± 0.5
8	8	76.4 ± 0.7	58.6 ± 0.7	9	76.9 ± 0.9	58.6 ± 0.7	8	77.4 ± 0.9	57.6 ± 0.7
9	7	83.9 ± 1.1	65.1 ± 1.0	8	83.4 ± 1.1	65.0 ± 1.0	9	84.3 ± 1.2	64.8 ± 0.9

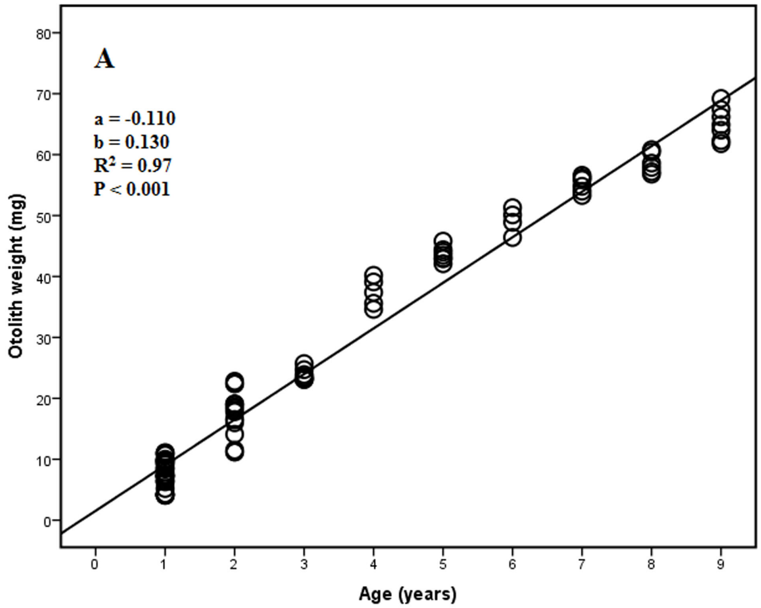
Variation around mean represents standard error.

TABLE 2

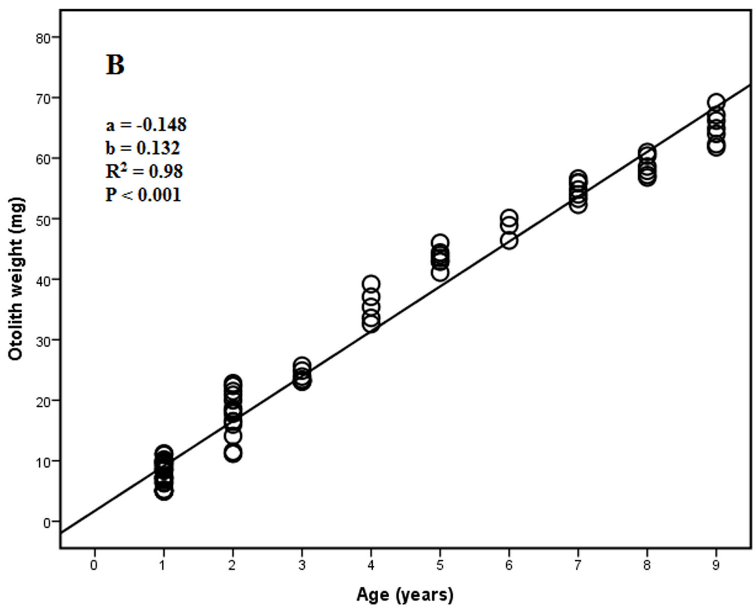
Estimated parameters of fish total length and otolith weight relationship of the three stocks of *Sperata aor* from the River Ganga

Stock	**n**	***a* (S.E.)**	***b* (S.E)**	**95 % CI of *a***	**95 % CI of *b***	**R^2**	**Sig.**
Narora-Kanpur	105	19.35 (0.349)	0.99 (0.010)	18.65-20.04	0.97-1.01	0.99	0.000
Varanasi	105	19.14 (0.360)	0.99 (0.011)	18.43-19.86	0.97-1.01	0.99	0.000
Bhagalpur	105	19.12 (0.360)	1.00 (0.011)	18.40-19.83	0.98-1.02	0.99	0.000

n= number of samples, *a*= intercept, *b*= slope, CI= confidence interval, R^2= coefficient of determination.



A
a = -0.110
b = 0.130
R2 = 0.97
P < 0.001
Otolith weight (mg)
Age (years)



B
a = -0.148
b = 0.132
R2 = 0.98
P < 0.001
Otolith weight (mg)
Age (years)

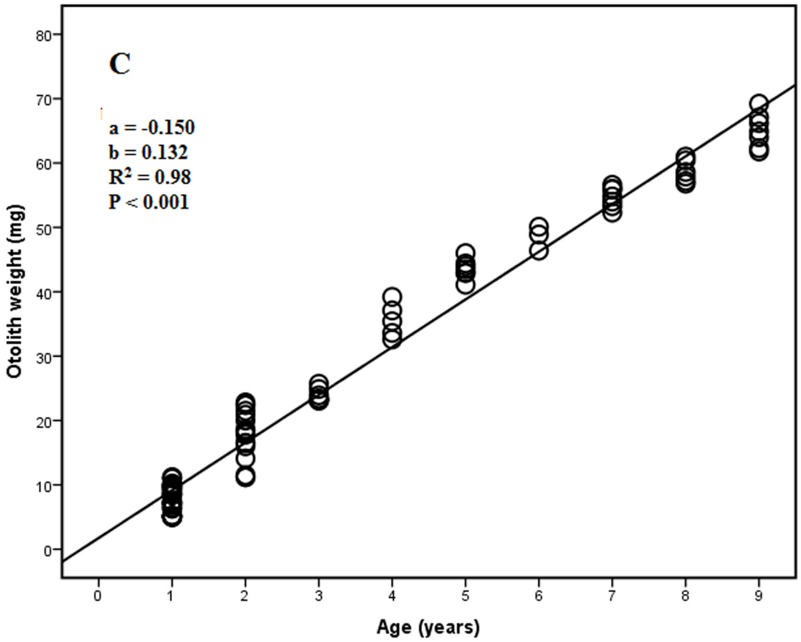
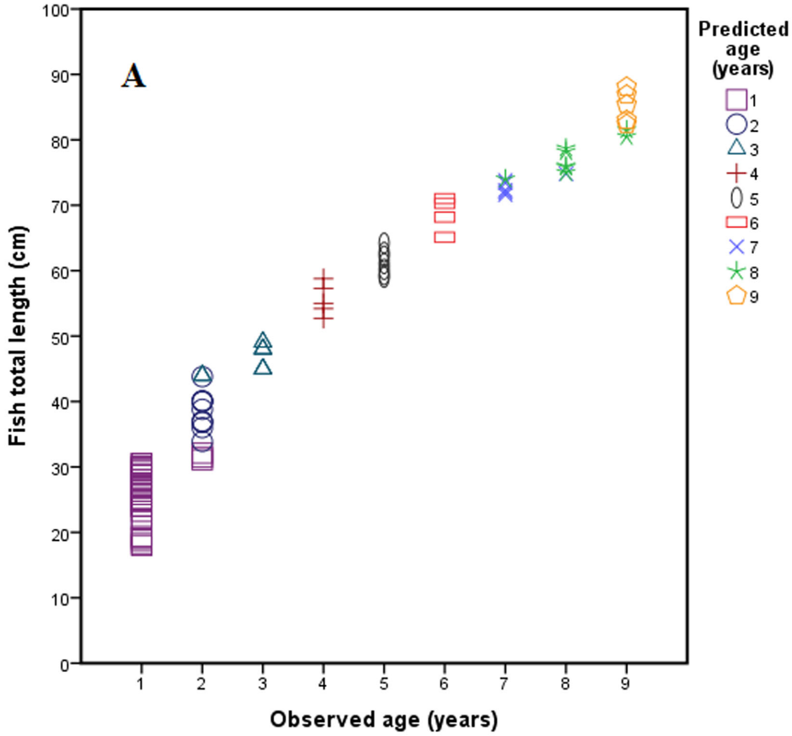
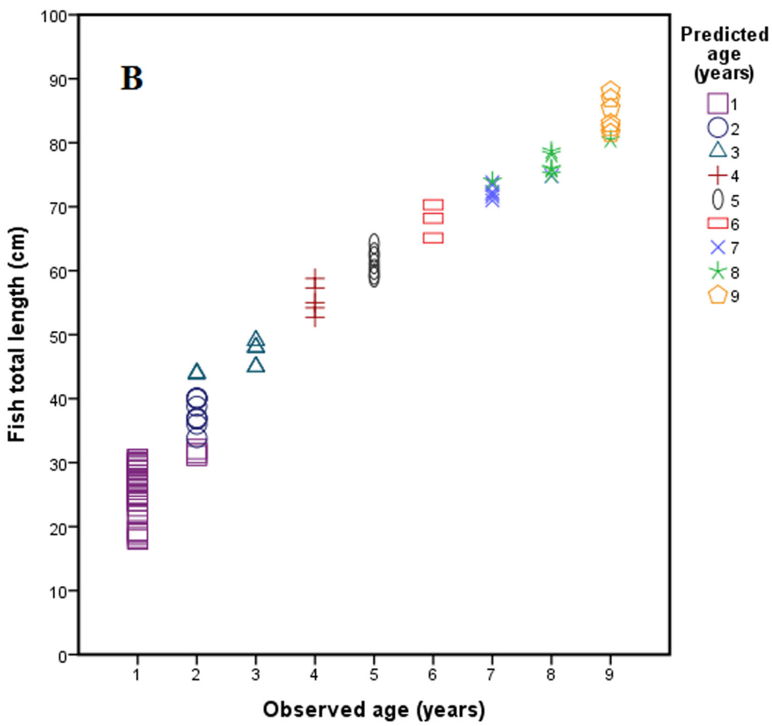


Fig. 1. Relationship between otolith weight and fish age for Narora-Kanpur **(A)**, Varanasi **(B)**, and Bhagalpur **(C)** stocks of *Sperata aor.*

The cross-validation classification method revealed 100 % of individuals of the Narora-Kanpur stock, Varanasi stock and Bhagalpur stock having predicted ages similar to the observed ages of 1, 3, 4, 5, and 6; 1, 3, 4, 5, and 6; 1, 3, 4, and 6, respectively. For age 2, 58.8 % of the individuals showed the same predicted age as the observed (remaining percentage predicted as age 1 and 3) in the three stocks. For age 5, 85.7 % of the individuals showed the same predicted age as the observed (remaining percentage predicted as age 6) in Bhagalpur stock. For age 7, 83.3 % of the individuals showed the same predicted age as the observed (remaining percentage predicted as age 8) in the Narora-Kanpur stock while in Varanasi and Bhagalpur stock correct classification was 85.7 %. For age 9, 71.4 % of the individuals showed the same predicted age as the observed (remaining percentage predicted as age 8) in all the selected stocks. Overall, 88.5, 88.8, and 87.2 % of the predicted ages were correctly classified to their observed ages for Narora-Kanpur, Varanasi, and Bhagalpur stock, respectively. The scatterplots showed the contribution of the predicted ages to the observed ages for the three stocks of *S. aor* (Fig. 2).


A
Fish total length (cm)
0
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5
6
7
8
9
Observed age (years)
Predicted age (years)
1
2
3
4
5
6
7
8
9


B
Fish total length (cm)
0
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5
6
7
8
9
Observed age (years)
Predicted age (years)
1
2
3
4
5
6
7
8
9

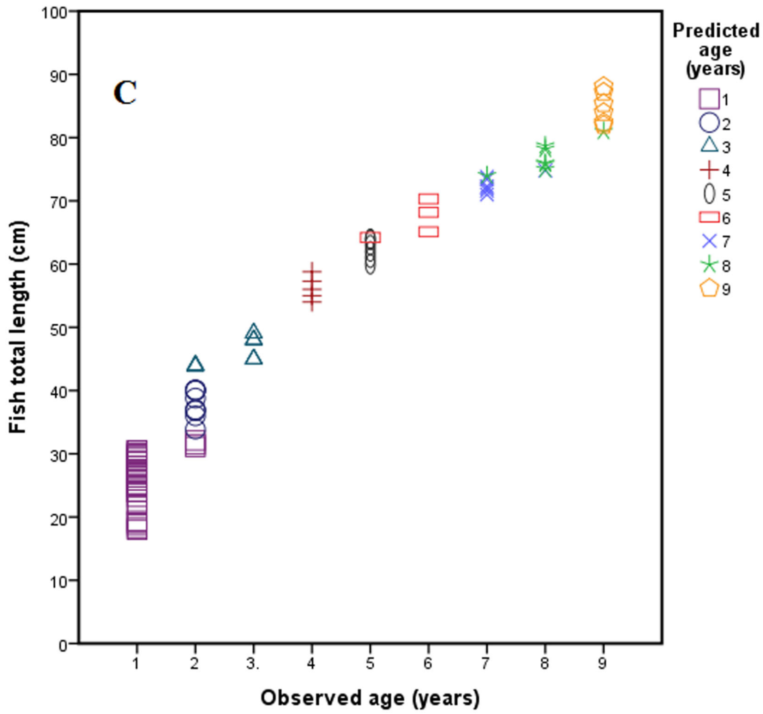


Fig. 2. Scatterplots showing contribution of predictive ages to the observed age for Narora-Kanpur stock **(A)**, Varanasi stock **(B)**, and Bhagalpur stock **(C)** of *Sperata aor.*

Discussion

The average weight of otoliths increased with age in the three stocks of *S. aor*, inferring that the otolith weight can be used as a quick and cost-effective substitute method for estimating age, as suggested in several published reports (Francis & Campana, 2004; Britton & Blackburn, 2014; Hanson & Stafford, 2017; Khan et al., 2018). Several of these studies have highlighted that average otolith weight continues to increase with age, unlike certain other variables e.g., fish length, otolith length, etc. It is reported that the otolith growth is different from somatic growth rate and may directly be related to time and age (Reznick, Lindbeck, & Bryga, 1989). Therefore, otolith weight may provide a more accurate estimate of age than the other variables and in some conditions may provide a reliable estimate of fish age than annuli counts of otolith (Worthington et al., 1995). For example, when there is some uncertainty in the interpretation of annuli especially in older fishes due to the curvature of the edge or the annuli being closely spaced along the otolith edge (Khan et al., 2016). In order to utilize the benefits of using otolith weight, the relationship between otolith weight and observed age should be calibrated, as the method is restricted by the reliability of the initial age estimates. That is, if imprecise estimates of age are used for calibration, the use of otolith weight to estimate age will be biased. Therefore, the use of known-age for calibration may significantly increase the reliability of subsequent age estimations using otolith weight (Lou et al., 2005).

Otoliths are primarily composed of calcium carbonate in the form of aragonite crystals to a fibroprotein organic matrix (Campana, 1999). Thus, the weight of otoliths is directly associated

with the amount of aragonite and the total organic matter. Moreover, the otolith chemical composition would affect the density of otolith and thus the weight of otoliths (Lou et al*.,* 2005). Consequently, a number of environmental factors such as chemical composition of ambient water, temperature and salinity should be further studied. The present study also provided an evidence of no significant variations in the otolith weight-age relationships among the selected stocks of the *S. aor* from the River Ganga. Few studies have reported significant variations in the otolith weight-age relationships at the spatial scale (Worthington et al., 1995; Lou et al., 2005).
The predictive model (discriminant function analysis) output showed greater than 85 % of estimated ages being correctly classified to their ages obtained from sectioned otoliths observed under the microscope in the three stocks of *S. aor*. Therefore, the output of the predictive model suggested that the estimated age obtained from sectioned otoliths were comparatively consistent with their relationship between total length and otolith weight (Britton & Blackburn, 2014). The accuracy of predictions of age does not vary significantly among the three stocks of the selected fish species. However, Lou et al. (2005) reported that the accuracy of predictions of age varied depending upon the spatial scale over which the prediction was made. We conclude that the relationship between otolith weight and fish age can be potentially utilized to estimate the age of the three stocks of *S. aor* from the river Ganga.

Acknowledgments

The authors are thankful to the Chairman, Department of Zoology, Aligarh Muslim University, Aligarh, India for providing necessary facilities for the study. We are also grateful to the Science and Engineering Research Board, Department of Science and Technology, New Delhi for funding the study and providing financial assistance to the first author (SR/SO/AS-40/2012).

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