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Morphometrics Analysis of Sagitta Otolith in Pool Barb, *Puntius sophore* (Hamilton, 1822)

Anju Rani[1] Deepak Rai[1] Anil K. Tyor[1]

[1] Department of Zoology, Kurukshetra University, Kurukshetra, Haryana, India

Address for correspondence Anju Rani, Research Scholar, Department of Zoology, Kurukshetra University, Kurukshetra 136119, Haryana, India (e-mail: thaakran2101@gmail.com).

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Abstract

Introduction The use of otolith morphometrics could prove to be a powerful tool in fish identification. The aim of the present study was to analyze the shape of the otolith in pool barb, *Puntius sophore*.

Materials and Methods To accomplish the present study, samples of various sizes were collected from the Yamunanagar and from the Faridabad fish markets in Haryana, India. The sagitta otoliths were extracted by making a horizontal cut across the head of the fish.

Results The independent *t*-test revealed no statistically significant difference between the values of otolith length and width of both the right and left otoliths ($p > 0.05$). Furthermore, various shape indexes, such as form factor (FF); circularity (C); rectangularity (REC), and aspect ratio (AR) were calculated, and the general shape of the otoliths of *P. sophore* was described as rectangular and less elongated. The otolith length (OL) was found to be positively correlated with the AR, whereas the FF was found to be negatively correlated with REC and C. The present study expresses the relationship between the total length (TL) and the head length (HL) of the fish with the OL and the otolith width (OW) by a linear regression model. The results depicted that the OL and the OW were linearly correlated to the TL and to the HL of the fish.

Conclusion The present study also provides a better understanding in identification of fish stock.

Keywords
- linear regression
- otolith dimensions
- shape indices

Introduction

Puntius sophore (Hamilton, 1822), commonly known as pool barb, stigma barb, and swamp barb, is a freshwater to brackish-water fish belonging to the cypriniformes order and to the cyprinidae family.[1] Cypriniformes are the largest group of fishes, with an estimated number of ~ 3,500 species.[2] *Puntius sophore* is widely distributed in inland waters of Asia, including Bangladesh, Pakistan, India, Nepal, Myanmar, Bhutan, Afghanistan, and China. This fish is benthopelagic (demersal), inhabiting rivers, streams, and ponds of plains.[3] It is considered as a chief food source for poor people in Bangladesh,[4] and is used as an aquarium fish.[5] According to the red list (2010) of the International Union for Conservation of Nature (IUCN), the status of this fish is regarded as of least concern.[6] However, studies from the Indian waters depicted that the fish is at lower risk to near threatened in the Western Ghat and in the Harike wetland, due to heavy fishing pressure.[7,8] Otoliths are paired calcified, aragonitic mineralizations located in the inner ear of the fish, which contribute to audition and equilibrium.[9,10] Amongst the three otoliths, the sagitta is the largest, followed by the astericus and by the lapillus.[11] The otolith continues to grow throughout the life of the fish, and its growth generally follows an allometric increase with respect to fish size.[12] The variations in the shape of the sagitta otolith are immense and are species specific, ranging from pinhead size to massive pieces of calcium carbonate ($CaCO_3$).[11] Due to its interspecific variations and larger size, the sagitta otolith has been used to estimate the taxon, age, size, migration, and feeding habits of fishes.[10,13,14]. Hence, knowledge of fish otolith morphometry is considered a valuable tool for the identification of the stock,[15,16] population management,[17] determination of diet

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Table 1 Variables utilized to study otolith morphology[22]

Variables	Measurements	Description
Relative dorsal length, (D)	d-d'	$D=\frac{d-d'}{l-l'}$ X 100
Relative medial length, (M)	m-m'	$M=\frac{m-m'}{l-l'}$ X 100
Relative antirostrum height, (A)	m-a	$A=\frac{m-a}{h-h'}X$ 100
Relative rostrum height, (R)	m-r	$R=\frac{m-r}{h-h'}$X 100
Relative antirostrum length, (AL)	al-d	$AL=\frac{al-d}{l-l'}$ X 100
Relative rostrum length, (RL)	rl-l	$RL=\frac{rl-l}{l-l'}$ X 100

in predatory fishes,[18] ontogenic research,[19] ecomorphological studies,[20] and for the identification of specific species.[21]

Although the otolith chemistry of marine fishes has been extensively studied, information on otolith of freshwater fishes concerning the Indian subcontinent is limited. Therefore, the purpose of the present study was aimed to analyze the shape and morphometrics measurements of the otolith of *P. sophore*.

Materials and Methods

A total of 41 specimens ranging between 66 and 109 mm in total length (TL) were procured from the fish markets of the Faridabad (28.4211° N and 77.3078° E) and of the Yamunanagar (30.133° N and 77.288° E) regions of Haryana, India, and brought to the laboratory in an ice box. All of the fish specimens were cleaned and measured for TL, standard length, HL, and body weight, nearest to 0.1 mm and 1 g, respectively. The sagitta otoliths were removed by making a horizontal cut across the head of the fish. The otoliths were cleaned manually by using 1% potassium hydroxide (KOH) solution to remove otic fluid, blood, and tissue, and were air dried. The right and left otoliths were kept separately in different labeled envelopes.

Table 2 Shape indices calculated using otolith morphometric parameters[23,24]

Parameters	Shape indices	Formulae
Area (Ar)	Aspect ratio (AR)	OL/OW
Perimeter (P)	Form factor (FF)	$4\pi Ar/P^2$
Otolith length (OL)	Rectangularity (REC)	Ar/OL/OW
Otolith width (OW)	Circularity (C)	P^2/Ar

Digital images of both the right and left otoliths were obtained over a dark background using a Magnus MSZ-TR stereo microscope (Magnus Analytics, New Delhi, India (fitted with a Magcam DCS 5.1MP, ½.5" CMOS SENSOR camera. Various morphometric measurements of the otoliths,[22] as shown in ►**Table 1**, were acquired using ProgRes CapturePro, version 2.80, software (Jenoptik AG, Jena, Germany), in which the otolith length (OL) was the maximum distance from the rostrum to the postrostrum, and the otolith width (OW) was the distance perpendicular to the length passing through the dorsal and ventral rim (►**Fig. 1**).

For the analysis of the shape of the otoliths, morphometric parameters such as OL, OW, area (Ar) and perimeter (P) were utilized to calculate four dimensionless shape indices (form factor (FF); circularity (C); rectangularity (REC); and aspect ratio (AR)[23,24] (►**Table 2**). Form factor is a mean to estimate the surface area irregularity, C gives information on the similarity of various features to a perfect circle, REC describes the variations of length and width with respect to the area, and AR expresses the shape tendency of the otolith.[23] To statistically analyze the data, SPSS for Windows, Version 16.0 (SPSS Inc, Chicago, IL, USA) and Microsoft Excel, version 2007 (Microsoft Corp., Redmond, WA, USA) were employed. The difference between the OL and the OW of the right and left otoliths was examined by employing the independent *t*-test. The relationship between the TL and the HL of the fish and the OL and OW was described by a linear equation.

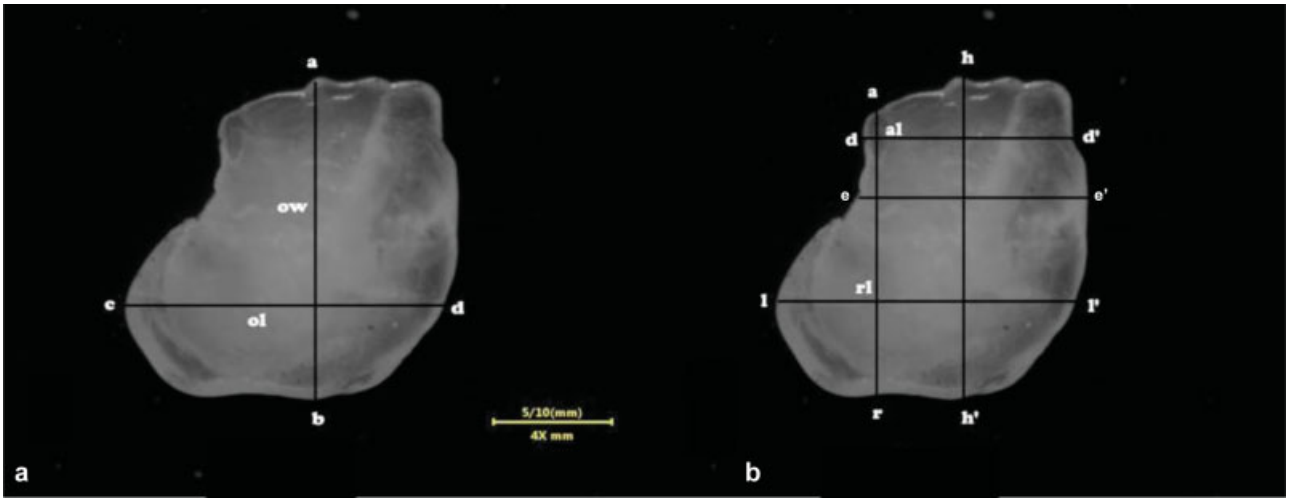

Fig. 1 Otolith of *Puntius sophore* (a) The distance between a and b is the otolith width, and the distance between c and d is the otolith length, (b) various otolith morphometric measurements used for the present study.

Table 3 Mean, standard deviation, standard error, minimum and maximum values of various body measurements of *Puntius sophore*

Parameter	Mean	SD	SE	Min	Max
Total length (TL)	83.9	10.4	1.6	66	109
Standard length (SL)	67.5	8.6	1.3	52	89
Head length (HL)	15	2.1	0.3	12	20
Body weight (BW)	104	36.9	5.8	40	210

Abbreviations: Max, maximum range; Min, minimum range; SD, standard deviation.
All of the values depicted in the table are in millimeters (mm)

Table 4 Mean, standard error, minimum and maximum values of various parameters of otolith dimensions

Parameters	Mean ± SE	Min	Max
Otolith length (OL)	0.74 ± 0.1	0.54	1.07
Otolith width (OW)	0.91 ± 0.0.2	0.61	0.98
Relative antirostrum height (A)	28.8 ± 1.68	14.03	65.74
Relative antirostrum length (AL)	8.51 ± 0.60	17.89	2.75
Relative rostrum height (R)	55.4 ± 2.05	18.10	75.79
Relative rostrum length (RL)	14.3 ± 1.25	4.53	33.27

Abbreviations: Max, maximum range; Min, minimum range; SE, standard error.
All the values depicted in table are in millimeters (mm).

Results

A total of 82 otolith samples were collected from 41 specimens. The OLs and OWs ranged between 0.54 and 1.07 mm, and between 0.61 and 0.98 mm, respectively. Various morphometric parameters of the fish were taken into consideration (►**Table 3**). The measurements of Otolith length (OL) and otolith width (OW) of both right and left otoliths were tested and no statistically significant difference was observed ($p > 0.05$). Therefore, either the left or right sagitta otolith can be used for the analysis. For the present study, the left sagitta otolith was utilized. The shape of the otolith of *P. sophore* was described as rectangular and less elongated, possessing well-defined antirostrum and rostrum. The antirostrum was observed as short and narrow, with average height and length of 28.8 ± 1.68 and 8.51 ± 0.6 0mm, respectively, whereas the rostrum was noticed to be wide and round with a mean height and length of 55.4 ± 2.05 and 14.3 ± 1.25 mm, respectively (►**Table 4**). The otolith has smooth dorsal and ventral margins with an obtuse excisural notch. The sulcus was found to be round and deep (►**Fig. 2b**). By comparing the mean values of 4 shape indices of the otolith of *P. sophore* (►**Table 5**), it was concluded that the average value of REC was the highest, while the value of AR was the lowest (AR: 0.82 < C: 1.36 < FF: 31.91 < REC: 34.03). Furthermore, the OL was found to be positively correlated with the AR, whereas the FF was found to be negatively correlated with REC and C. The present study explains the relationship between the TL and the HL of the fish with the OL and the OW described by the linear equations $y = 0.0052x + 0.309$ (►**Fig. 3a**); $y = 0.0095x + 0.1114$ (►**Fig. 3b**); $y = 7.2851x + 9.5285$ (►**Fig. 3c**), respectively. The results depicted that the OL and the OW were linearly correlated to the TL and to the HL of the fish.

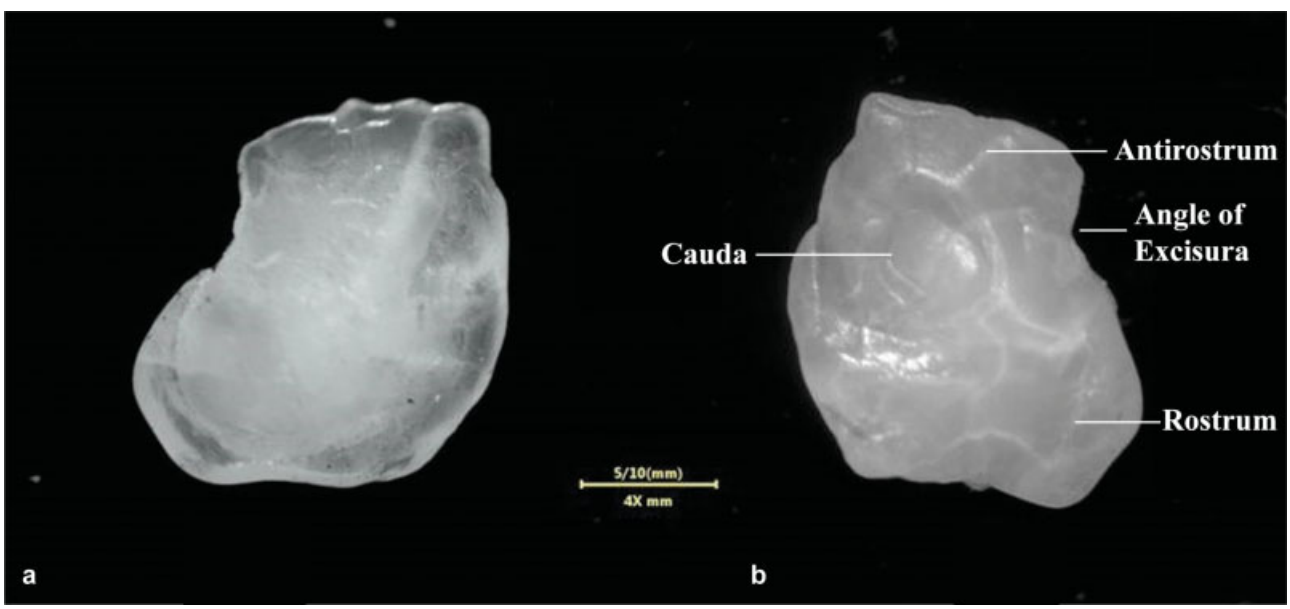

Fig. 2 *Puntius sophore* otolith (a) dorsal view of the left sagitta otolith, (b) ventral view of left sagitta otolith showing the cauda, the rostrum, the antirostrum, and the angle of excisura.

Table 5 Descriptive statistics of shape indices of *Puntius sophore*

Shape indices	Mean	SD	SE	Min	Max
Form factor	31.91	5.20	0.81	0.31	34.57
Aspect ratio	0.82	0.1	0.02	0.61	0.98
Rectangularity	34.03	2.57	0.40	28.52	39.86
Circularity	1.36	3.93	0.61	2.35	27.72

Abbreviations: Max, maximum range; Min, minimum range; SD, standard deviation; SE, standard error.

The OW was found to be a better parameter in estimating fish length than the OL.

Discussion

Otolith morphology has proven to be a powerful and vital tool in various taxonomic studies. Among the three otoliths, the sagitta otolith has been extensively utilized in various taxonomic studies related to age, growth, feeding habits, and stock identification, due to its larger size and great interspecific variability.[10,13,14,25] The present study has aimed to examine the relationship of fish TL and HL with otolith dimensions (OL and OW) by a linear regression model. The otolith dimensions (OL and OW) and fish body relationships have been studied in various marine fish species by linear regression models.[15,26–32] The results of the present study depicted that the OL and the OW were linearly correlated to the TL of the fish. The HL of the fish also showed positive correlation with the OL. The OW was found to be a better parameter than the OL in estimating fish length. Hence, it is suggested that otolith dimensions increase as fish length increase and, therefore, otolith growth can be correlated with fish growth. These results are similar to previous studies.[33,34]. However, other studies depicted that the relationship of otolith variables and fish somatic growth are not necessarily linear.[35,36] In studies on the relationship between otolith and fish size, the OL was usually used.[15,26,37–39] The present study supplies supplementary information by considering both the OL and the OW, as well as the HL of the fish. The present study also described various other morphometric parameters to give a detailed observation of the shape of the otolith. When comparing the values of the OL and of the OW of both right and left otoliths, no statistically significant difference was observed, which was consistent with the previous findings of different authors.[29,32,35,40] But some studies of sciaenid fishes, such as *Micropogonias furnieri* and *Macrodon ancylodon*, and of teleost fishes, such as *Lycodes palearis* (Zoarcidae) revealed inverse findings.[15,41] Concerning the findings of the present study, it becomes evident that the knowledge of the otolith morphometrics is considered an important marker in the identification of species and in many other ecological studies that aimed to determine the prey size based on otoliths obtained from the stomach contents of piscivorous predators, because, when the relationship between the OL and the TL in a

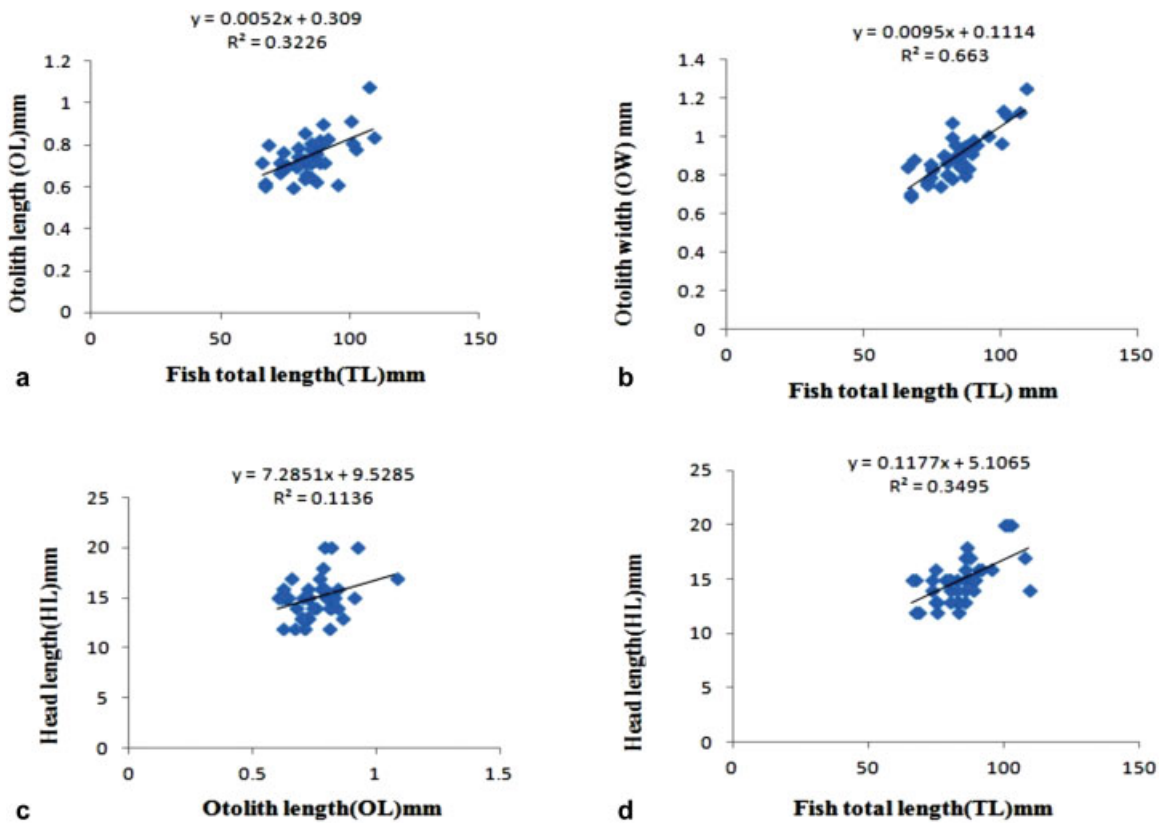

Fig. 3 Relationship between (a) otolith length and fish total length, (b) otolith width and fish total length, (C) head length and otolith length, and (d) head length and fish total length.

species is determined, the TL or standard length of a fish can be easily estimated from its OL, or vice versa.[28,31,33] The present study also provides a better understanding in the identification of the stock.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Acknowledgments

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