

Comparative Otolith Morphology and Morphometry of Cyprinid Fishes from Indian Waters

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Abstract—Asteriscus, the largest otolith in the members of Cypriniformes was utilized to explain the inter-specific variability among four cyprinids i.e. *Labeo rohita* (Hamilton, 1822), *Cirrhinus mrigala* (Hamilton, 1822), *Catla catla* (Hamilton, 1822) and *Cyprinus carpio* (Linnaeus, 1758). To accomplish the study n parameters characterizing the Morphology and Morphometry of otoliths were estimated [n = number of the parameters], various otolith dimensions such as otolith length (OL), width (OW) and six shape descriptors i.e. Form factor (FF); Circularity (C); Rectangularity (REC); Aspect-ratio (AR); Ellipticity (E) and Roundness (RD) were calculated. Significant difference was observed in the shape and otolith dimensions viz. OL, OW, Area, Perimeter. Six shape indices were also found to be statistically significant. Relationship of otolith length (OL) and otolith width (OW) with fish total length (TL), standard length (SL) and head length (HL) were expressed by linear regression model. coefficient of determination (R^2) revealed that OL (otolith length) is more related with fish total length (TL) than width. This information could be used to determine the fish size or vice a versa. Moreover, the shape variation in otolith could provide a better understanding of fish identification.

Keywords: Asteriscus, otolith, Cypriniformes, shape indices

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INTRODUCTION

Cyprinids, the most diversified group of the fresh water ichthyofauna consists of about 3500 species worldwide (Nelson, 2016). Members of the family have a wide range of habitat and geographical distribution (Nelson, 2016). This family includes various commercially important species. Because of their exceptional adaptive abilities and wide geographical range, cyprinids play a significant part in the food chain of natural freshwater bodies and in commercial fish ponds. With such fundamental significance, studies have begun to focus on their diversity.

The fishes are identified on the basis of their morphological characteristics like color patterns, body contour, fin formula etc. but, occasionally, these morphological characters may become indistinct. Hence, there is an urgent need to explore the alternate characters which can validate the morphological characters and give more credibility to the identification procedure. Fish possess various hard parts such as otolith, scales, the 1st dorsal fin spine/ray or anal fin spine, vertebrae, operculum and pharyngeal teeth etc. Of these hard structure otoliths were extensively studied by fish biologist because of their species-specific characteristics.

Otoliths are the paired calcified structures in the form of aragonite embedded in the protein matrix located in the fish inner ear (Campana, 2004). The

inner ear of teleosts possesses three otoliths namely, sagitta, asteriscus and lapillus (Popper et al., 2005). Sagitta acts as a mechanoreceptor whereas, Asteriscus serves the function of sound reception and lapillus concerned in maintaining equilibrium (Popper et al., 2003). In majority of fishes, sagitta is the largest otolith and asteriscus is the smallest one (Paxton, 2000; Tuset et al., 2008). However, in the members of Cypriniformes and Siluriformes, the asteriscus is the largest, whereas, sagitta is the smallest otolith (Harvey et al., 2000; Assis, 2003; Campana, 2004).

Morphological characters of otolith displayed species specificity that range from simple disc shape of some flatfish (Pleuronectidae) to the irregular shape of others such as redfish. These variations in the otolith morphology are related to the different environmental factor such as temperature and depth of water (Monteiro et al., 2005). Due to this species specificity, otolith has been extensively utilized as a good tool in the assessment of the stock (Begg and Brown 2000). Otolith structure also provides information about the age of its bearer (Ilkyaz et al., 2011). Analysis of morphological and morphometric characters of otolith plays a significant role in the identification of various fish population (Lombart et al., 1991).

Despite vast diversity of the cyprinids, majority of work have been documented on marine fishes. But the research on Indian freshwater fishes regarding Otolith

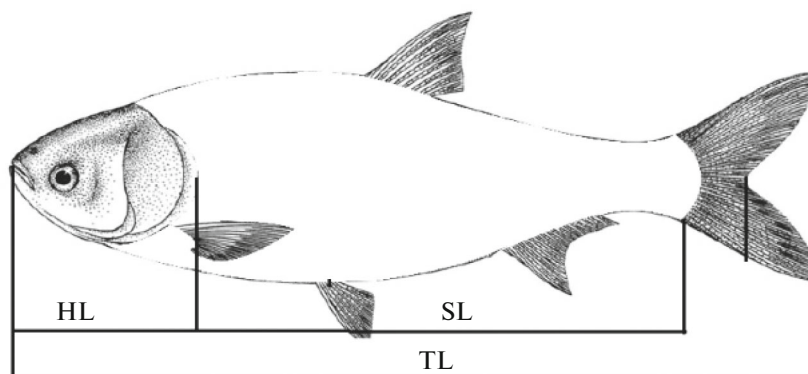


Fig. 1. Morphometric Measurements of Fish HL—Fish head length, SL—Fish standard length, TL—Fish total length.

morphology is scarce so, the present investigation aimed to focus on the otolith morphology and morphometry of four cyprinid fish i.e. *Labeo rohita* (Hamilton, 1822), *Cirrhinus mrigala* (Hamilton, 1822), *Catla catla* (Hamilton, 1822) and *Cyprinus carpio* (Linnaeus, 1758) from Indian waters.

MATERIALS AND METHODS

The fish specimens were procured from local fish markets of Delhi and Haryana and brought to the laboratory in ice box. Taxonomic position and number of specimens of each species is depicted in (Table 1). Fish samples were cleaned thoroughly with water and measured for different body measurements like total length of the fish (TL), standard length (SL), head length (HL) and body weight (BW) nearest to 0.01 cm and 0.01 g. Otoliths present in the grooves beneath the brain were removed by making horizontal cut across the head (Fig. 2). Thereafter, otoliths were cleaned manually with the help of fine brush using 1% KOH solution to remove otic fluid, blood and tissue, consequently air dried. Both right and left otolith were kept separately in different plastic bags with the reference code of the fish entitled correctly over it for further analysis. Digitalized images of both the otoliths were recorded under dark background using “MSZ-TR” stereo microscope fitted with Magcam DCS 5.1MP,1/2.5” CMOS SENSOR camera. To accomplish the study 2 otolith parameters characterizing the otoliths morphometry were estimated. n parameters characterizing the morphology of otoliths were esti-

mated (Fig. 1) (n = number of the parameters and please draw a measurement scheme—Fig. 1). The parameters were Otolith length (OL) the longest distance between the most anterior and posterior points and otolith width (OW)—the longest distance between the ventral and dorsal edges were recorded using the “ProgRes capturePro” version 2.80 software (Fig. 3). Additionally, various shape descriptors i.e. Form factor (FF); Circularity (C); Rectangularity (REC); Aspect-ratio (AR); Ellipticity (E) and Roundness (RD) were also calculated following (Tuset et al., 2003) and (Lord et al., 2012) (Table 2).

The data obtained on morphometric parameters was subjected to statistical analysis using SPSS version (16.0) 16. The difference between right and left otolith and were analyzed using paired t -test. ANOVA (Analysis of variance) was used to determine any statistical difference between various shape indices of otolith in different species. The relation of the fish total length (TL) with otolith length and width (OL and OW) were depicted by regression equation.

In section Materials and Methods, it is necessary to mention the use of correlation analysis to assess the relationships between the parameters under consideration. And also please specify in which statistical package the calculations were made—Statistics 6.

RESULTS

A total of 344 asteriscus otoliths were removed from 172 specimens of four species (*L. rohita*, *C. mrigala*, *C. catla* and *C. s carpio* belonging to two different

Table 1. Taxonomic position and number of specimens of selected species

Subfamily	Species	No. of specimens
Labeoninae	<i>Labeo rohita</i> (Hamilton, 1822)	44
	<i>Cirrhinus mrigala</i> (Hamilton, 1822)	50
Cyprininae	<i>Catla catla</i> (Hamilton, 1822)	30
	<i>Cyprinus carpio</i> Linnaeus, 1758	48



Fig. 2. Showing the location of otolith in the head region of the fish.

subfamilies i.e. Labeoninae and Cyprininae from Delhi and Haryana with total length ranging from 15–30 cm. The study revealed that otolith of fish displayed excellent inter-specific variations in both morphology and morphometry. Morphologically, otolith showcased diversity in its shape from oval to round rectangular to kidney-shaped. Besides shape, some variations in other morphological parameters such as margin, excisural notch, sulcus opening, shape of rostrum and anti-rostrum were also observed.

Otolith Morphology

***Labeo rohita*.** The otolith of *L. rohita* varied from round to rectangular in shape with serrated margins. Anterior margin was wide and concave while posterior margin was crenulated. Lateral and medial margins were oblique and less serrated with well-defined antero-lateral, Antero-medial and posterior-medial edges. The excisural notch was wide and obtuse. Sulcus was heterosulcoid with pseudo-ostial opening which was devoid of well-defined ostium and cauda. Rostrum appeared to be broad and rounded in contrast to antirostrum (Fig. 3a).

***Cirrhinus mrigala*.** The shape of otolith of *C. mrigala* was oval with irregular margins. Anterior margin was rounded while posterior was oblique and medial margin was slightly serrated. Otolith of *C. mrigala* had prominent and extended antero-medial and antero-lateral and posterior-lateral edges. Excisural notch was wide and obtuse similar to *L. rohita*. Sulcus was heterosulcoid with ostial opening and defined ostium and

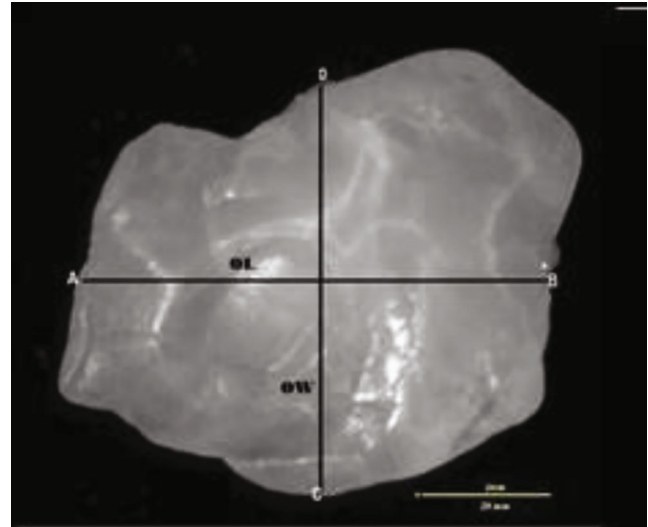


Fig. 3. Showing otolith measurements: (A, B) otolith length (OL), (C, D) otolith width (OW).

cauda. Rostrum was broad, round and elongated whereas, antirostrum was short and pointed (Fig. 3b).

***Catla catla*.** The shape of otolith of *C. catla* was oval with irregular margins. Anterior margin was broad and rounded while posterior was oblique with less pronounced edges. Sulcus was heterosulcoid with mesial opening with less defined ostium and cauda. Excisural notch was wide and obtuse. Rostrum was broad and massive while, antirostrum was short and rounded (Fig. 3c).

***Cyprinus carpio*.** The shape of otolith of *C. carpio* was kidney shaped with lobed and undulated margins.

Table 2. Shape indices and their formulae

Shape indices	Formulae
Aspect-ratio (AR)	OL/OW
Form-factor (FF)	$\frac{(4\pi A)}{P^2}$
Circularity (C)	$\frac{P^2}{A}$
Rectangularity (REC)	$\frac{A}{\left(\frac{OL}{OW}\right)}$
Roundness (RD)	$\frac{(4A)}{\pi(OL)^2}$
Ellipticity (E)	$\frac{(OL - OW)}{(OL + OW)}$

Abbreviations: OL—otolith length, OW—otolith width, P—perimeter of otolith, A—area of otolith, AR—Aspect ratio, FF—Form factor, C—circularity, REC—Rectangularity, RD—Roundness, E—Ellipticity.

Table 3. Various otolith dimensions in different fish species

Species	OW	OL	Area	Perimeter
<i>L. rohita</i>	1.24 ± 0.03	1.44 ± 0.04	1.59 ± 0.07	4.82 ± 0.12
<i>C. mrigala</i>	1.34 ± 0.04	1.63 ± 0.04	1.90 ± 0.08	5.48 ± 0.13
<i>C. catla</i>	1.15 ± 0.05	1.41 ± 0.05	1.34 ± 0.08	4.51 ± 0.16
<i>C. carpio</i>	1.18 ± 0.02	1.53 ± 0.04	1.55 ± 0.05	5.11 ± 0.08

For designation see Table 2. All the values are Mean ± S.E. (Standard Error) of mean.

Table 4. Various otolith shape indices in different fish species

Species	AR	FF	C	REC	RD	E
<i>L. rohita</i>	1.174 ± 0.013	0.893 ± 0.057	16.344 ± 1.107	0.839 ± 0.011	0.911 ± 0.011	0.079 ± 0.006
<i>C. mrigala</i>	1.229 ± 0.025	0.78 ± 0.006	16.158 ± 0.128	0.857 ± 0.013	0.898 ± 0.015	0.097 ± 0.01
<i>C. catla</i>	1.241 ± 0.029	0.813 ± 0.012	15.543 ± 0.241	0.815 ± 0.02	0.841 ± 0.019	0.104 ± 0.012
<i>C. carpio</i>	1.325 ± 0.025	0.74 ± 0.013	17.28 ± 0.359	0.846 ± 0.01	0.826 ± 0.017	0.135 ± 0.009*

For Designation of parameters.

Anterior portion of the otolith was roughly concave while posterior was oblique and with less distinct edges. Sulcus of the otolith was heterosulcoid with pseudo-ostial opening and defined ostium and cauda. Excisural notch was deep and obtuse. Rostrum was broad, elongated and rounded in contrast to antirostrum which was noted as short and rounded (Fig. 3d).

Analysis of Otolith Morphometry

In addition to morphology, otolith morphometry also revealed significant differences among species.

The difference between the length and width of right and left otolith was found statistically insignificant (paired *t*-test, $p > 0.05$). Hence, either of the two otoliths could be used for morphometric analysis. In the present study, left otolith was preferred. Statistically significant difference of each of the considered species from the other three ones was observed in the otolith length (OL), otolith width (OW), area and perimeter of four cyprinids (ANOVA, $p < 0.05$) (Table 3). Moreover, six dimensionless shape indices viz. aspect ratio, form factor, circularity, rectangularity, roundness and ellipticity were also calculated to explain the inter-spe-

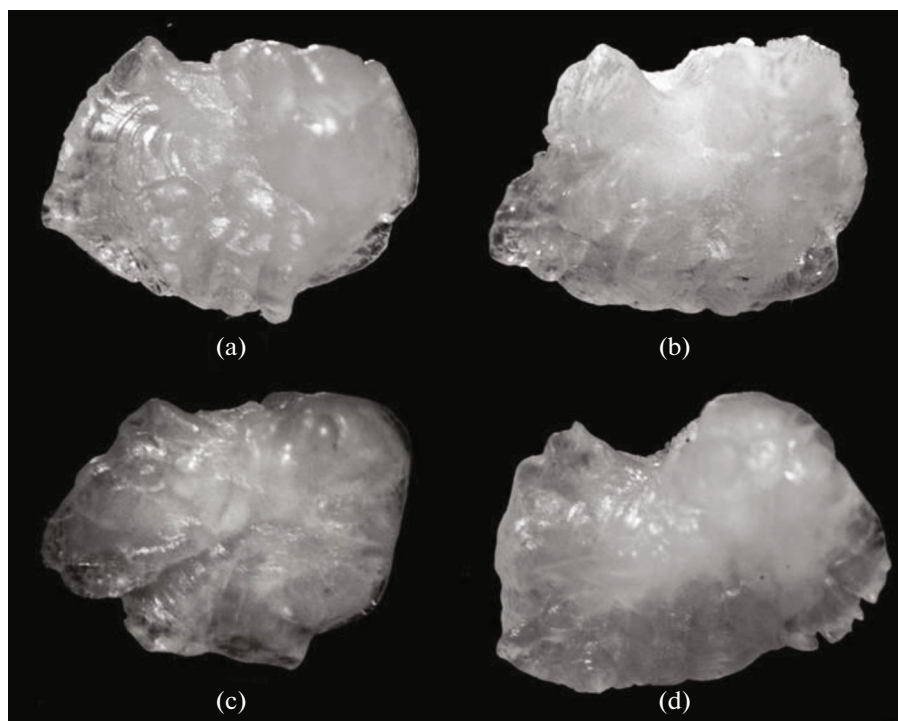


Fig. 4. Showing otolith shape in four cyprinids fish (a) *L. rohita*, (b) *C. mrigala*, (c) *C. catla* and (d) *C. carpio*.

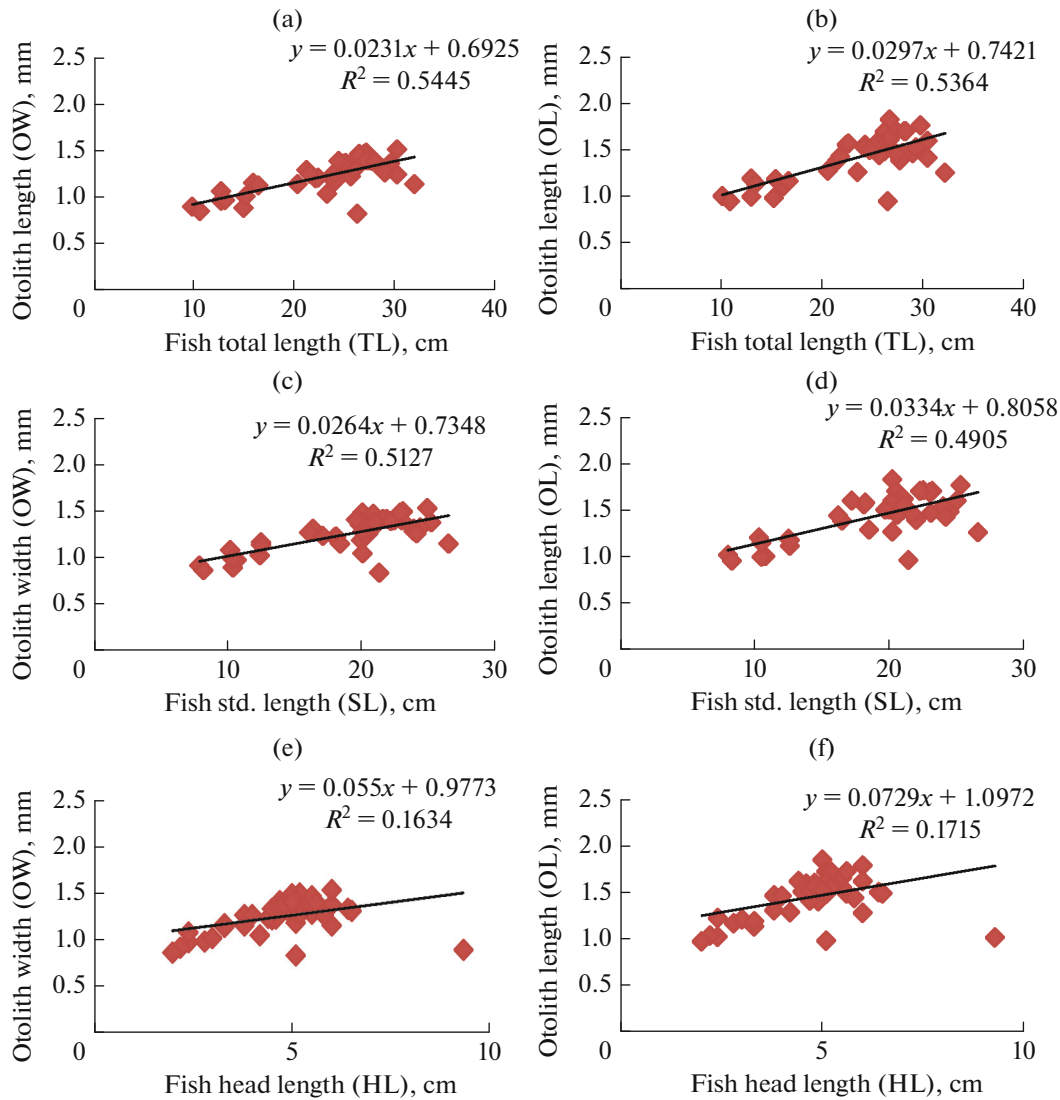


Fig. 5. Relationship of otolith length and width with total length, standard length and head length of *L. rohita* (a) otolith width with total length, (b) otolith length with total length, (c) otolith width with standard length, (d) otolith length with standard length (e) otolith width with head length (f) otolith length with head length of the fish.

cific variability. Significant differences in the shape indices among the four cyprinids were observed when comparing any pair of species (ANOVA, $p < 0.05$) (Table 4). The relationship of otolith dimensions (OL and OW) with fish total length (TL), Standard length (SL) and head length (HL) was established by regression model (Fig. 5). Study showed linear relationship of otolith dimensions with TL, SL, and HL. The value of coefficient of determination (R^2) showed that fish total length (TL) was found to be more related with otolith length (OL) than otolith width (OW) (Table 5).

DISCUSSION

Otoliths are paired; hard calcareous structure that resides in the fish inner ear and aid in equilibrium and

audition (Popper and Lu, 2000; Campana, 2004; Popper et al., 2005). The otoliths present in the three otolithic organs viz., the saccule, lagena and utricle (Popper and Lu, 2000). Species-specific property of otolith has documented by various authors (Tuset et al., 2003; Ponton, 2006; Bani et al., 2013; Ferri et al., 2018). The present investigation revealed immense inter-specific variability of otolith in its shape and sulcus opening but little variability was observed in other morphological features such as type of sulcus, appearance of cauda and ostium, shape of rostrum and antirostrum. Our results showed versatility in shape of otolith that varied from oval to rectangular to kidney shaped among four cyprinids (Figs. 3a–3d). Heterosulcoid type of sulcus was noted in all four cyprinids. Variation in sulcus opening was observed in the present study, Pseudo-ostial opening

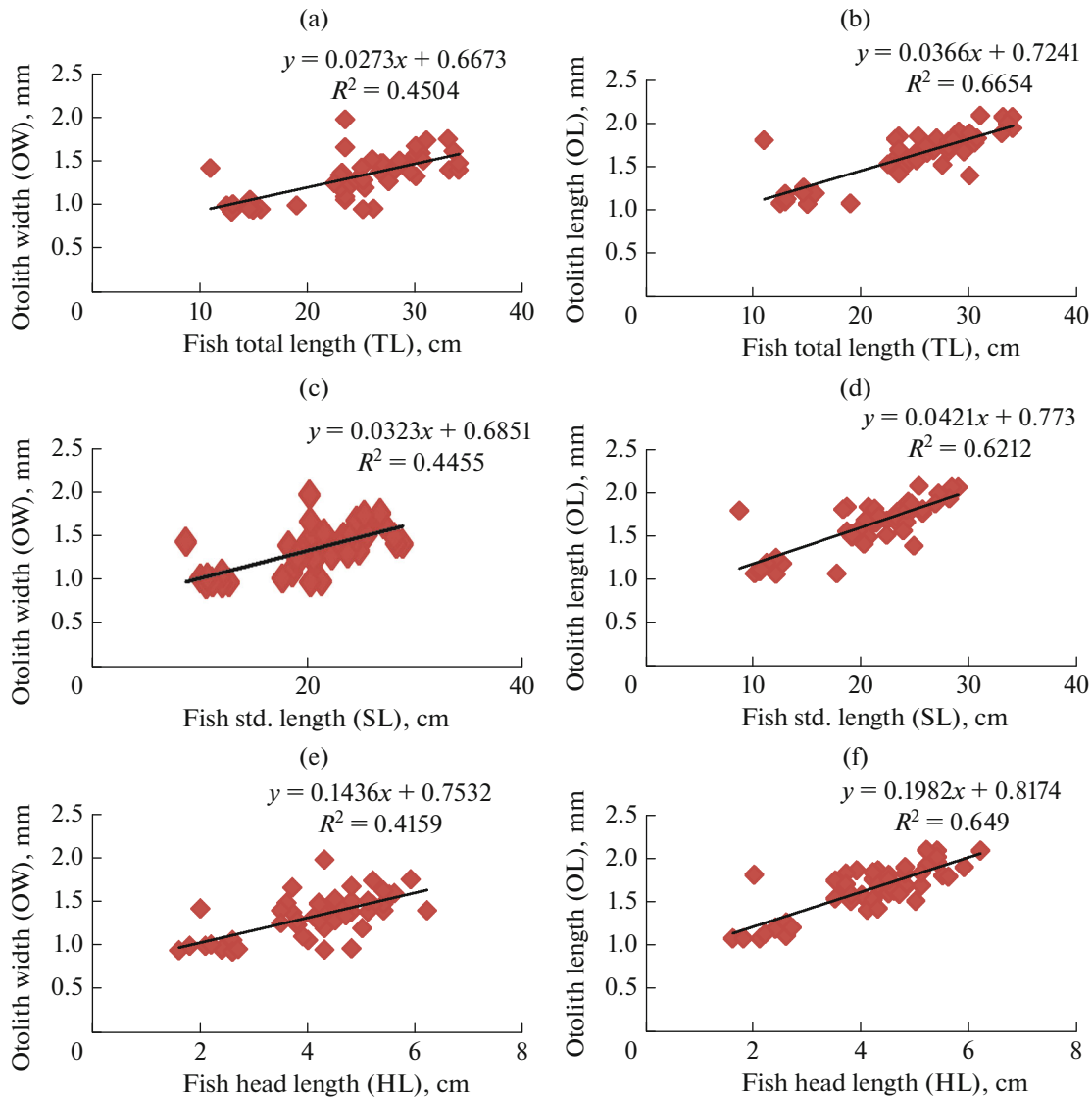


Fig. 6. Relationship of otolith length and width with total length, standard length and head length of *C. mrigala* (a) otolith width with total length, (b) otolith length with total length, (c) otolith width with standard length, (d) otolith length with standard length (e) otolith width with head length (f) otolith length with head length of the fish.

was recorded in *L. rohita* and *C. carpio*, ostial type of sulcus opening was observed in *C. mrigala* and mesial type of sulcus was found in *C. catla*. Well-defined ostium and cauda were observed in *L. rohita*, *C. mrigala* and *C. carpio*. Similar study on morphological variation in otolith was reported by Jawad et al. (2007) and Jawad (2008) on *Saurida tumbil* and triplefin (*Enneapterygius* spp). This morphological variability in the otolith might be due to the different habitat of the fish, food supply, varied temperature and depth of the water in which they are residing.

Besides morphology, otolith biometry also contributes significantly in the field of fisheries research. During the present study, analysis of otolith length (OL) and otolith width (OW) of the four cyprinids spe-

cies showed that otolith of *C. catla* was smallest among the four species (Table 3). Moreover, the differences in six shape indices i.e. aspect ratio, form factor, circularity, rectangularity, roundness and ellipticity were found to be statistically significant ($p < 0.05$) among the species which signifies inter-specific variability (Table 4). The mean values of roundness, circularity, roundness and ellipticity revealed irregularities of otolith surface. But the study of Zening et al. (2015) did not show significant difference in all six shape indices. Analysis of the shape indices could be helpful in determining the regional differences in otolith morphology (Tuset et al., 2003).

Most of the studies mainly focused on relationship of otolith length and width with fish size only (Harvey

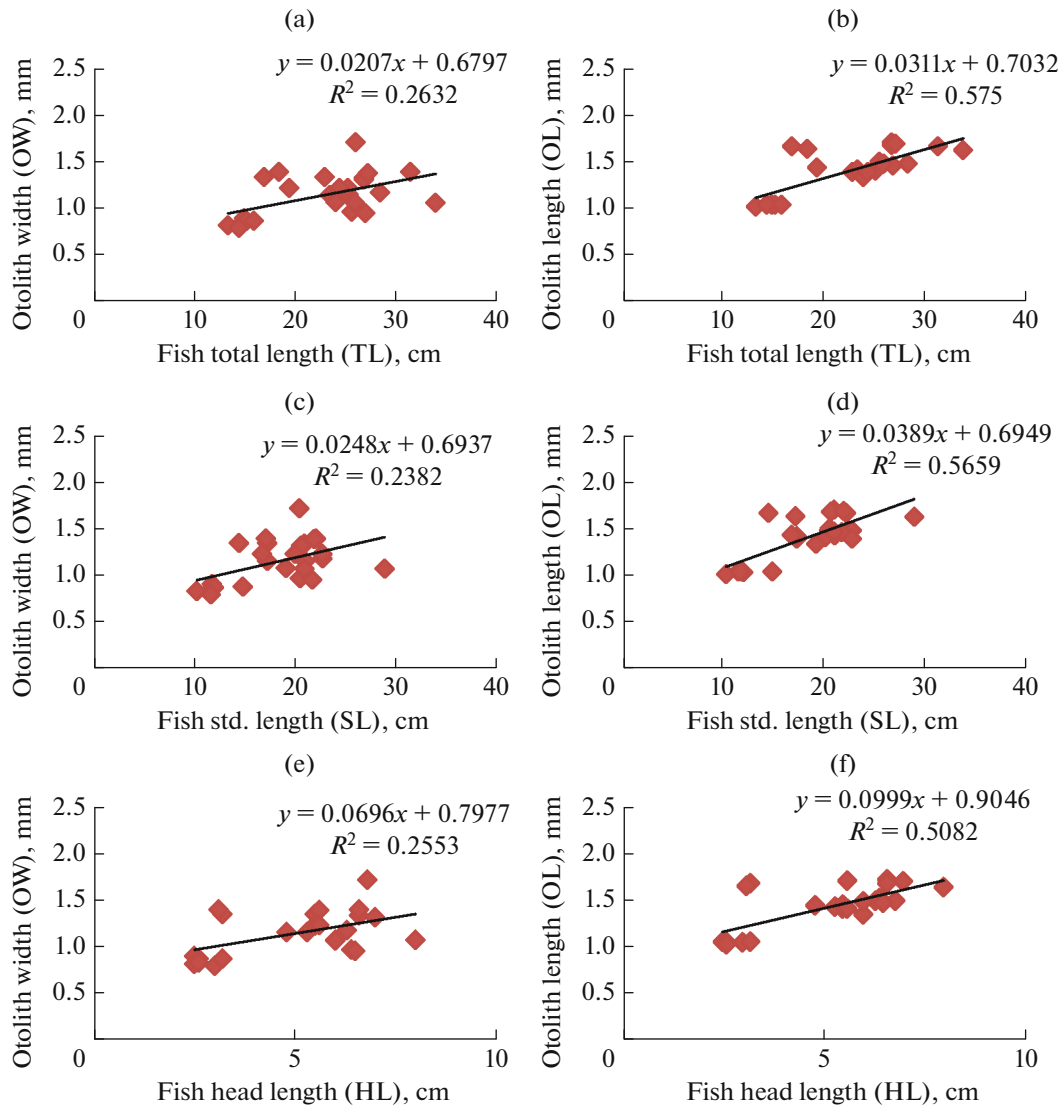


Fig. 7. Relationship of otolith length and width with total length, standard length and head length of *C. catla* (a) otolith width with total length, (b) otolith length with total length, (c) otolith width with standard length, (d) otolith length with standard length (e) otolith width with head length (f) otolith length with head length of the fish.

et al., 2000; Waessle et al., 2003; Battaglia et al., 2010). But the present study provides the additional information on relationship of otolith dimensions with fish head length (HL).

The relationship between fish total length (TL) and otolith dimensions provides valuable information that could aid in determining the fish length and vice-versa that assists in analyzing the digestive content of the predator animals. Since otolith did not get eroded while passing through the digestive system of the predator it could also help in estimating the actual size and species of prey fish (Bostanci, 2009). The current investigation depicted linear relationship of otolith dimensions (length and width) with fish head length (HL), total length (TL) and standard length (SL)

(Figs. 4–7). Results of the present study also revealed strong relationship of fish head length (HL) with otolith dimensions which signifies that as the fish head length increases otolith length also increases. Analyzing the value of coefficient of determination (R^2) it was observed that strongest relationship was noted between fish total length (TL) and otolith length (OL) whereas, moderate relationship was observed among total length (TL) and otolith width (OW) (Table 5). The findings of the present study were similar to the work of Basusta et al. (2013) and Altin and Ayyilidiz (2017). Strong correlation between fish size and otolith length showed influence of somatic growth on otolith growth (Munk, 2012).

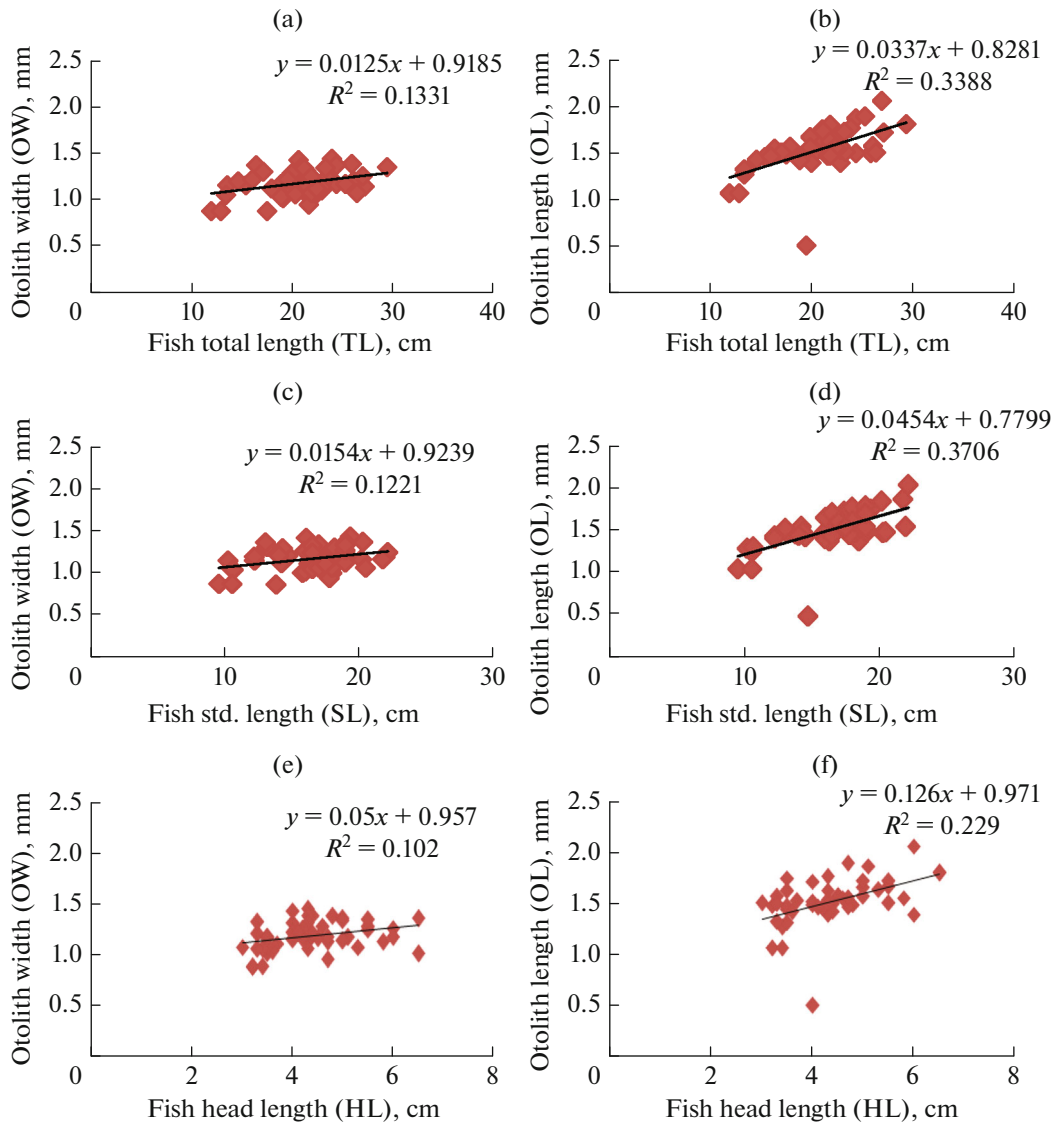


Fig. 8. Relationship of otolith length and width with total length, standard length and head length of *C. carpio* (a) otolith width with total length, (b) otolith length with total length, (c) otolith width with standard length, (d) otolith length with standard length (e) otolith width with head length (f) otolith length with head length of the fish.

CONCLUSIONS

Monitoring of the morphology and the morphometric analysis is crucial in estimating the size and the dietary need of the fish. Relation between fish size and otolith dimensions provide valuable information that may help in determining the fish length and vice-versa that assists in analyzing the digestive content of the predator animals.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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