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Relationship between fish body and sagittal otolith morphometrics in the pool barb *Puntius sophore* (Hamilton, 1822) from the Ganga and Punpun rivers of Bihar, India

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Abstract

The present study estimated the morphometric relationships between body and sagittal otolith of the economically and nutritionally important pool barb *Puntius sophore* (Hamilton, 1822). A total of 266 fish specimens were collected monthly from the Ganga and Punpun rivers of Bihar, India from September 2021 to March 2022. Length-weight relationships (LWRs), length frequency distribution, condition factor and otolith aspect ratio were assessed. The fish length and weight ranged from 5.57 to 11.17 cm and 1.18 to 18.98 g, respectively. The *b* value in LWRs (b>3) were estimated as 3.20 and 3.17 for Ganga and Punpun, respectively. Condition factors indicated optimal growth conditions for this species in both the rivers. The fish and otolith morphometrics showed a positive correlation, and the highest coefficient of determination (R^2) was observed between total fish length and otolith weight (R^2=0.942) followed by fish length and otolith length (R^2=0.90). All of these relationships are significant to fisheries management and useful for future time series studies on food-feeding habits, stock structure, environmental change and resource conservation.

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Introduction

Puntius sophore (Hamilton, 1822), is a small native fish species often known as "pool barb,"soft fin swamp barb" and "pothi", belonging to the family Cyprinidae and inhabits both fresh and brackishwaters such as rivers, streams, ponds, beels, floodplains and submontane regions (Froese and Pauly, 2022). This species is surface-pelagic, herbivore and shoaling in nature and is widely distributed throughout the Indian subcontinent, including India, Nepal, Afghanistan, China, Bangladesh, Bhutan, Myanmar, and Pakistan (Hossain *et al.,* 2018; Sarkar *et al.,* 2019). This is an economically important ornamental fish as well as food fish, and is also considered as a key target species in recreational fisheries and forms a major component of the tropical fish trade (Gupta *et al.,* 2018). Nutritionally, it is an important source of protein, carbohydrates, and micronutrients, and it helps to prevent malnourishment in people from rural areas (Hossain *et al.,* 2018). Mahanty *et al.* (2014) revealed high concentrations of the essential amino acids (histidine; 22.94±0.01%), unsaturated fatty acid (oleic acid 28.63±0.02%), and low fat (5%). The mineral profiles also showed that calcium (9,748.2 ±23.96 mg kg^{-1}) is the most abundant macro-mineral, followed by potassium (2,283.7±12.75 mg kg^{-1}) and sodium (1,610±22.34 mg kg-1). It also contains a lot of micro-minerals such as Fe (115.0±8.81 mg kg^{-1}), Zn (51.1±10.15 mg kg^{-1}) and Mn (11.2±2.09 mg kg^{-1}).

According to the CAMP report this is under Lower Risk Near Threatened category and listed in the International Union for the Conservation of Nature (IUCN) Red List as "Least Concern" (CAMP, 1998; Dahanukar, 2010). At present, the population of *P. sophore* in Indian rivers is steadily declining, and it is now considered a near threatened species due to irregular overfishing, habitat destruction, pollution and other climate or environmental changes (Dua and Prakash, 2009; Rani *et al.,* 2019; Sarkar *et al.,* 2019).

Information on the morphometrics and other life history characteristics of *P. sophore* is essential to comprehend the existing stock structure in various habitats and to manage and conserve them sustainably in their native ecosystem (Hossain *et al.,* 2009; Prajapati *et al.,* 2022). Several authors have studied the life history traits including length-weight relationship, length frequency distribution, condition factor, sexual maturity and mortality parameters of this species (Reddy and Rao, 1992; Hossain *et al.,* 2009, 2012; Hossain, 2010; Ahamed *et al.,* 2012; Pal *et al.,* 2013; Saroniya *et al.,* 2013; Gupta *et al.,* 2018; Ahirwal *et al.,* 2022). Reddy and Rao (1992) and Hossain *et al.* (2018) have shown isometric to positive allometric growth of *P. sophore* in river, pond, lake, and wetland ecosystems. However, Srivastava and Singh (2003) as well as Latif *et al.* (2017) revealed negative allometric growth of this species. Sarkar *et al*. (2019) observed length at first maturity within the size range of 4.4-12.6 cm for this species.

Fish otoliths, calcium carbonate (aragonite) structures located in the inner ear of fish, have significance in investigating the life history, stock, environmental influence and conservation status of any fish species (Tuset *et al.*, 2008; Kumar *et al.*, 2012; Chanthran *et al.*, 2021). All bony fishes have three pairs of otoliths, namely, *Sagitta*, *Astericus,* and *Lapillus,* and among these, Sagitta is the largest in most of the fishes. It shows species-specific variations in shape, size, and it grows throughout the life of the fish and hence, it is the most commonly used hard part for various studies on age, eco-morphology, stock identification, ontogeny, palaeontology, migration patterns and feeding habits of fish (Carpenter *et al.*, 2003; Tuset *et al.*, 2008; Eroglu and Sen, 2009; Gerard and Malca, 2011).

In terms of biodiversity and taxonomy, the genus *Puntius* is of great importance. Majority of research on *P. sophore* to date focused on morphometry, food-feeding habit, reproductive biology, and nutritional aspects. Various authors have documented dissimilar growth parameters for *P. sophore*, from which is quite evident that much more extensive research is required to fill up the knowledge gaps. This species is known to have a small otolith, and only very few publications are available about this species' otolith morphology. To address this insufficient information about fish and otolith morphometrics of *P. sophore*, an in-depth study was undertaken. The results of this study would form baseline information for future research on age, growth, stock structure, and biomass for the conservation and management of the species.

Materials and methods

Sampling

The sampling sites for *P. sophore* were Digha Ghat (25° 39' 11.959" N; 85° 5' 39.535" E) and Raj Ghat (25° 29' 30.843" N; 85° 01' 10.671" E) of the Ganga and Punpun rivers of Bihar, India (Fig. 1). Fish samples were taken from landings harvested employing different kinds of traditional fishing gears such as drift gill net (mesh size 10-20 mm, length 80-100 m, width 1.5-2 m) and barrier net (*Khairel Jaal*) (cod end mesh size 5-10 mm, cod end width 1-1.5 m, length 6-8 m, width 8-10 m) during September 2021 to March 2022. Fresh samples were stored in ice immediately after collection and brought to the laboratory for further studies. A total of 266 specimens were collected, of which 148 were from the Ganga River ranging in size from 5.59 to 11.01 cm, and 118 from the Punpun River ranging in size from 5.56 to 11.17 cm.

Morphometric measurements

The total length (TL, cm) and body weight (BW, g) were initially measured for each specimen to the nearest 1 mm using a digital vernier caliper and 0.01 g using an electronic balance, respectively. The sagittal otoliths were then removed from the fish head using the "up through the gills method' and no preservative was used prior to extraction (Secor *et al.*, 1992). A total of 532 otoliths were collected from 266 specimens. The damaged otoliths were discarded, and fresh otoliths were cleaned with water to get rid of tissue, blood, and fluids. They were then allowed to air dry and stored in separate vials. Each otolith was photographed in the laboratory using stereo-microscope (Olympus CX33) fitted with Magcam DC-5. Both the left and right otoliths were considered. For the analysis of otolith morphometrics, the following measurements were obtained from each specimen: otolith length (O_L, cm), the greatest distance between the anterior-most and posterior-most edges; otolith width (OW, cm), the longest distance from the dorsal to the ventral margin; and the weight of the sagittal otolith (Owt, g). The left and right otolith with otolith morphology is illustrated in Fig. 2. The otolith aspect ratio is calculated as the ratio between the length and width of the otolith and this ratio expresses the shape tendency of the otolith.

Data analysis

Based on the results of paired t-test, the difference between left and right sagittal otolith was found to be insignificant. Therefore, the data of only one otolith (left one) were used in this study. The relationship between fish size (TL, BW) and otolith size (O_L, Owt) was determined for each site using linear and exponential regression model (Le Cren, 1951). The length-weight relationship (LWR) was assessed using the equation $W = a \times L^b$, where W is body weight (BW,

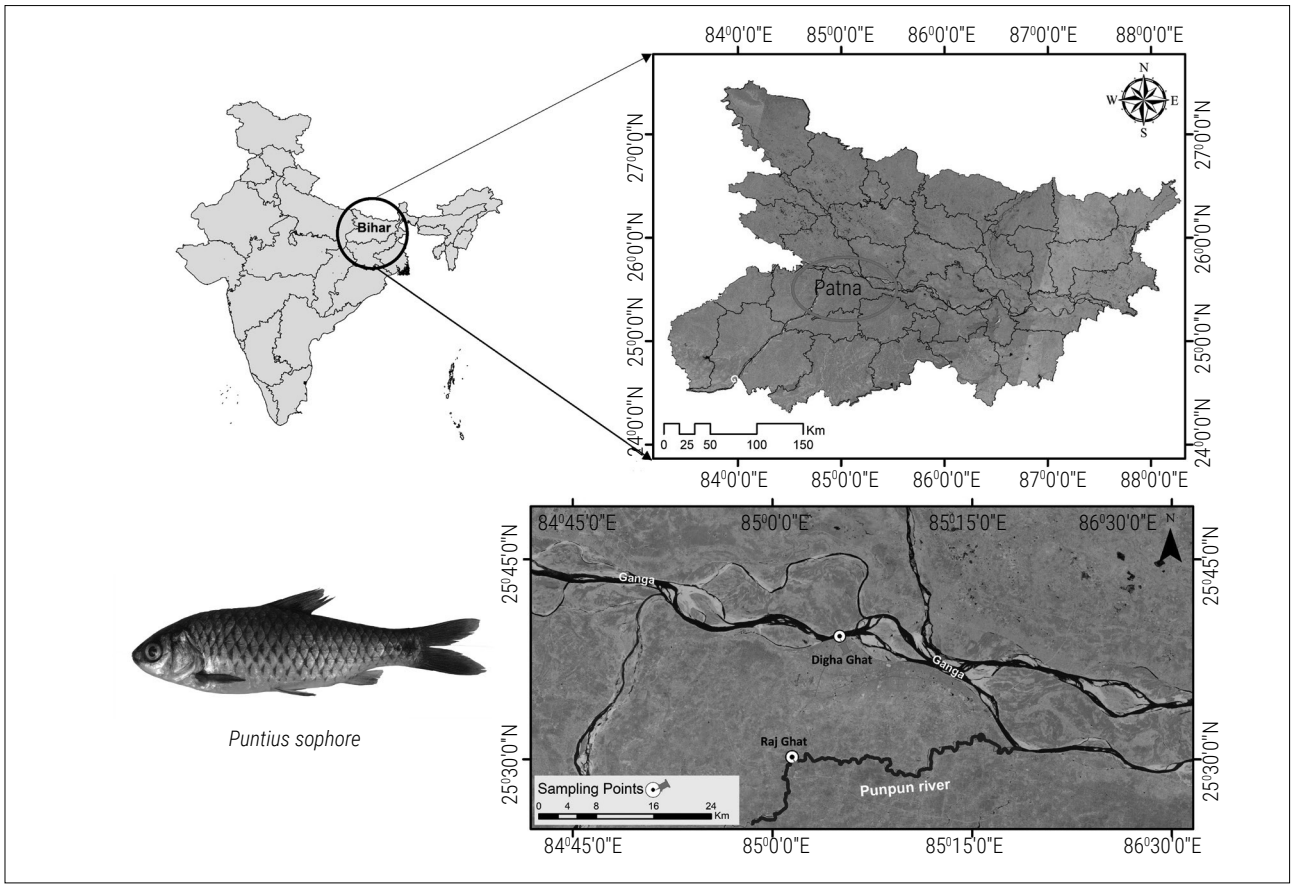

Fig. 1. Map showing sampling locations of *P. sophore* from the Ganga and Punpun rivers of Bihar, India

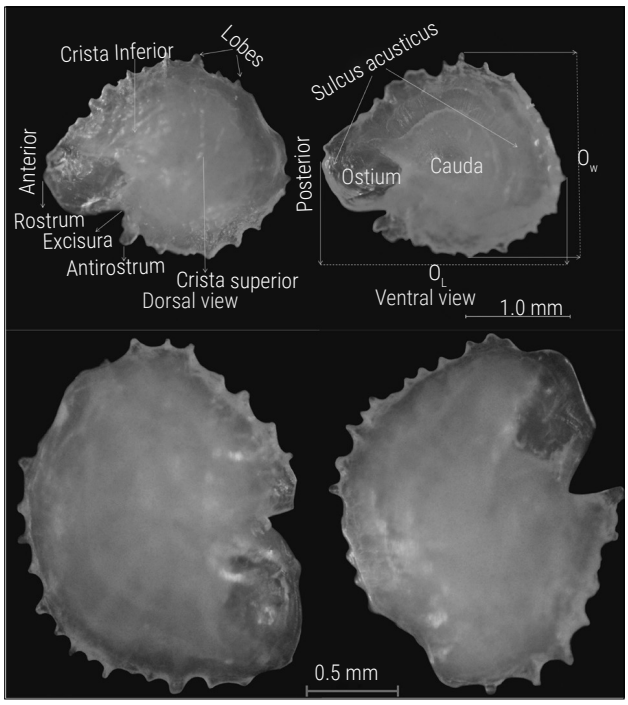

Fig. 2. (a) Otolith morphology and (b) Left and right sagittal otoliths of *P. sophore*

g) and L is the total length (TL, cm). Parameters *a* and *b* were estimated from the intercept and slope of the linear regression analysis based on the log-transformed length and weight data; log (*W*) = log (*a*) + *b* log (L). The coefficient of determination (R^2) was also estimated to evaluate the strength of each relationship. The Fulton's condition factor (K_F) and relative condition factor (K_n) were calculated using the equation; $K_F = 100x (W/TL^3)$ and $K_n = Wo/Wc$, respectively; where W is body weight (BW, g), Wo is observed weight, Wc is calculated weight and TL is total length (TL, cm) (Le Cren, 1951). All the statistical data analyses were performed using Microsoft Excel 2016 and Past 4.03 software. The presence of outliers was determined according to Froese *et al.* (2011).

Results

Length-weight relationship (LWRs) and condition factor of fish

A total of 266 fish specimens of *P. sophore* with an average length of 7.35 cm and a weight of 5.20 g were examined, and the descriptive statistics on length-weight measurements

with a 95% confidence limit are illustrated in Table 1. A maximum total length of 11.17 cm and a weight of 18.98 g were obtained from the Punpun River. The length and weight frequency distributions for both locations revealed that the group with a length of 6-8 cm and a weight of 1.25-6.25 g from the Ganga River accounted for 70-75% of the total population, while a comparable population coverage was obtained in wider range from the Punpun River with a length of 6-8.8 cm and weight 1.25-7.5 g (Fig. 3).

The LWRs of *P. sophore* from the Ganga and Punpun rivers were validated using the equations log W = log 0.0079 + 3.20 log L and log W = log 0.0076 + 3.17 log L, respectively,

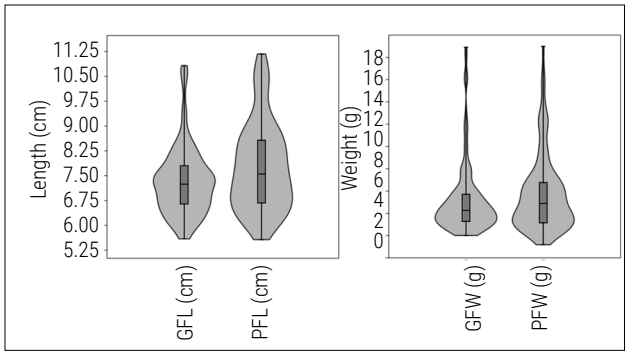

Fig. 3. Length and weight frequency distribution plot of *P. sophore* from the Ganga and Punpun rivers. GFL/GFW: Fish length/weight from the Ganga River; PFL/PFW: Fish length/weight from the Punpun River

for the pooled data. The LWR of *P. sophore* from both rivers exhibited positive allometric growth based on the regression coefficient values *b* (*b*>3.0), indicating optimal growing circumstances for this species. The LWRs of *P. sophore* from both locations were highly significant (p<0.001) and had a high coefficient of determination (R^2>0.902). The LWRs of *P. sophore* is illustrated in Fig. 4.

The condition factors (K_A Allometric, K_F Fulton's, and Kn Relative) from both the locations are presented in Table 2. Mann Whitney U-test revealed a significant difference (p<0.05) between the KF and KA values of the Ganga and Punpun rivers. In addition, the unpaired t-test showed that the KF values were significantly different (p<0.001). In contrast, the Mann Whitney U-test for Kn values, was insignificant (p=0.833; U=8382).

Otolith length-weight relationship

The descriptive statistics and length-weight relationship of the total 266 sagittal otoliths of *P. sophore* from both locations are given in Table 3. The mean length of the sagitta from the Ganga and Punpun rivers were observed to be 1.41±0.03 and 1.71±0.39 mm, respectively, and the highest length of 2.81 mm and weight of 6.65 mg were observed from the Punpun and Ganga rivers, respectively. The LWRs of otolith from the Ganga and Punpun rivers showed coefficients of determination of (R^2) 0.768 and 0.915, respectively. The LWRs of both locations are

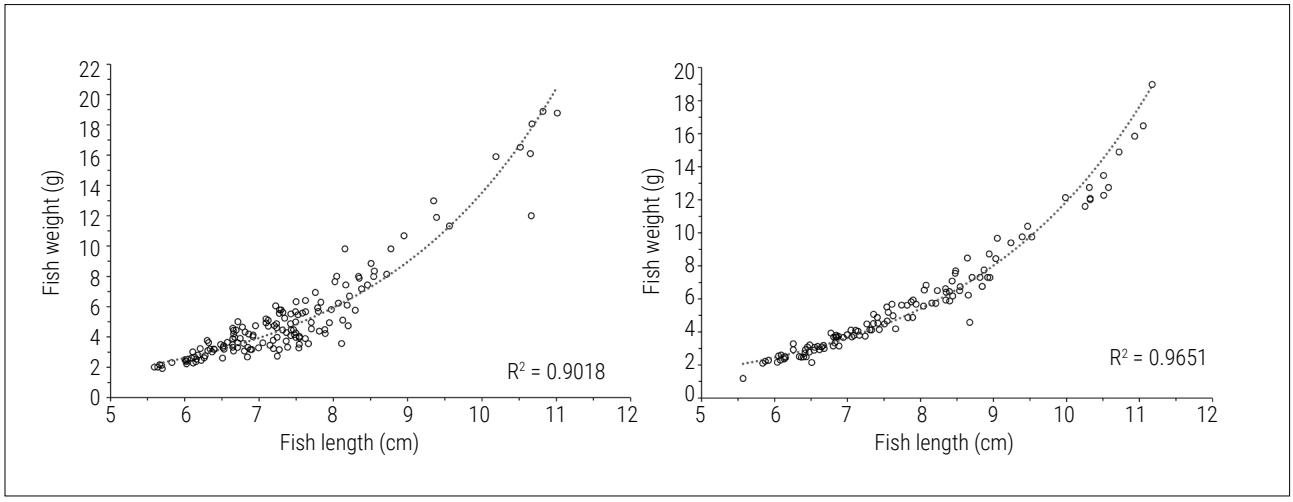

Fig. 4. Length-weight relationship of *P. sophore* from different rivers of Bihar, India. (a) Ganga River; (b) Punpun River

Table 1. Descriptive statistics and length-weight equation for morphometrics of *P. sophore* from different rivers of India

Location	Sex	n	Measurement	Mean±SE	Min.	Max.	95%CL	Equation	R^2	b
Ganga	C	148	TL (cm)	7.35 ± 0.09	5.59	11.01	7.17-7.52	Log w = log 0.0079 + 3.20 log L	0.902	3.20
			BW (g)	5.19 ± 0.27	1.93	18.89	4.66-5.74			
Punpun	C	118	TL	7.66 ± 0.13	5.57	11.17	7.52-8.01	Log w = log 0.0076 + 3.17 log L	0.960	3.17
			BW	5.72 ± 0.32	1.18	18.98	5.08-6.36			

Table 2. Condition factors (K_A, K_F, K_n) of *P. sophore* from the Ganga and Punpun rivers of Bihar, India

location	Sex	n	Condition factors	Mean±SD	Min.	Max.	95%CL
Ganga	C	148	K_A	0.008 ± 0.001	0.004	0.011	0.0078-0.0083
			K_F	1.201 ± 0.216	0.668	1.809	1.165-1.235
			K_n	1.010± 0.178	0.551	1.489	0.982-1.039
Punpun	C	118	K_A	0.007 ± 0.0008	0.003	0.010	0.0075-0.0078
			K_F	1.100 ± 0.123	0.457	1.360	1.077-1.123
			K_n	1.053± 0.344	0.382	2.250	0.989-1.116

Table 3. Descriptive statistics and length-weight equations for otolith dimensions

Location	n	Measurements	Mean±SD	Min-max	95% CL	Length-weight equation	R^2	95% CL of b	O_{AR}
Ganga	148	OL (mm)	1.41±0.03	1-2.54	1.36-1.49	log (W)= 0.713+1.77 log(L)	0.768	1.72-1.81	1.099±0.160
		Owt (mg)	4.13±0.74	2.79-6.65	4.01-4.25				
Punpun	118	OL (mm)	1.71±0.39	1.18-2.81	1.64-1.79	log (W)= 0.829+1.71 log(L)	0.916	1.55-1.71	1.084±0.182
		Owt (mg)	4.39±0.72	3.28-6.62	4.26-4.52				

given in Fig. 5. The otolith shape tendency was determined using the otolith aspect ratio (O_{AS}), and the highest value of 1.099 ±0.16 (Mean±SD) was recorded in the samples of Ganga River. The aspect ratio data from both the rivers confirmed to a normal distribution (Kolmogorov-Smirnov, p>0.05).

Fish and otolith morphometric relationship

Using linear and exponential regression models, the association between fish length-otolith length, fish length-otolith weight, and fish weight-otolith length of Ganga and Punpun river samples were determined. Fish length and otolith length of Ganga and Punpun rivers showed coefficients of determination (R^2) values of 0.867 and 0.903, respectively (Fig. 6). Similarly, fish length and otolith weight showed a substantial association, with samples from the Punpun River exhibiting the greatest coefficient of determination (R^2) of 0.9422 (Fig. 6). All relationships between fish length and otolith morphometrics were found to be significantly different (p<0.05).

Discussion

Length-weight relationship and condition factor

The morphometric characteristics of fish and otolith of the small native species *P. sophore,* with length-weight frequency, length-weight relationship, condition factor, fish otoliths, and fish morphology relationships from the Ganga and Punpun river systems of India are exceptionally rare, and few studies have been conducted in India. Due to the selectivity of the fishing gear and market preferences, it was impossible to collect specimens smaller than 5.57 cm in length and 1.18 g in weight (pooled data) during the study (Hossain *et al.,* 2012). The maximum length and weight of *P. sophore* were observed at 11.17 cm and 18.98 g, respectively, and a broader range of length-weight frequency was also obtained from the Punpun River. The maximum length obtained was shorter than the recorded value of 18 cm from India

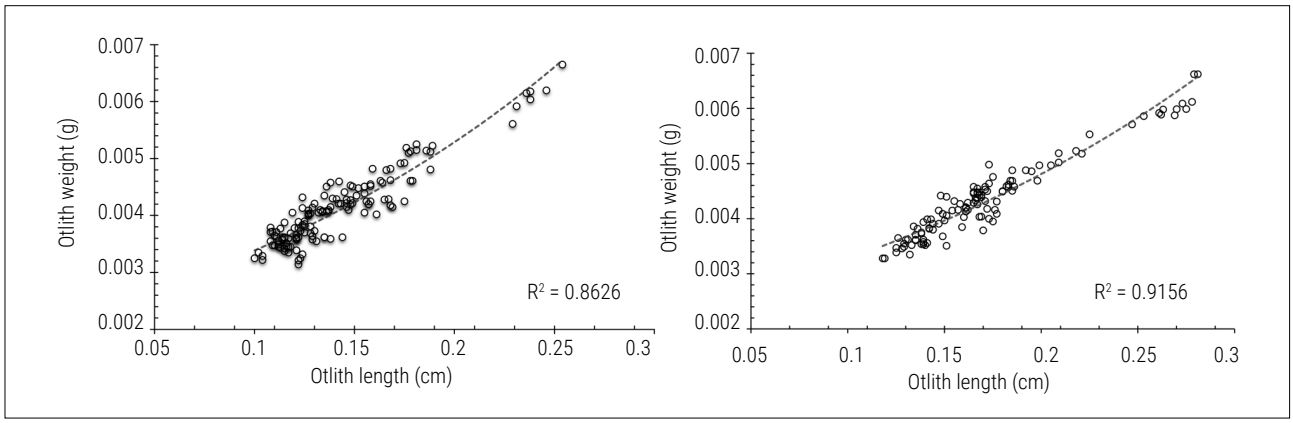

Fig. 5. Length-weight relationship of *P. sophore* otolith from different rivers. (a) Ganga River; (b) Punpun River

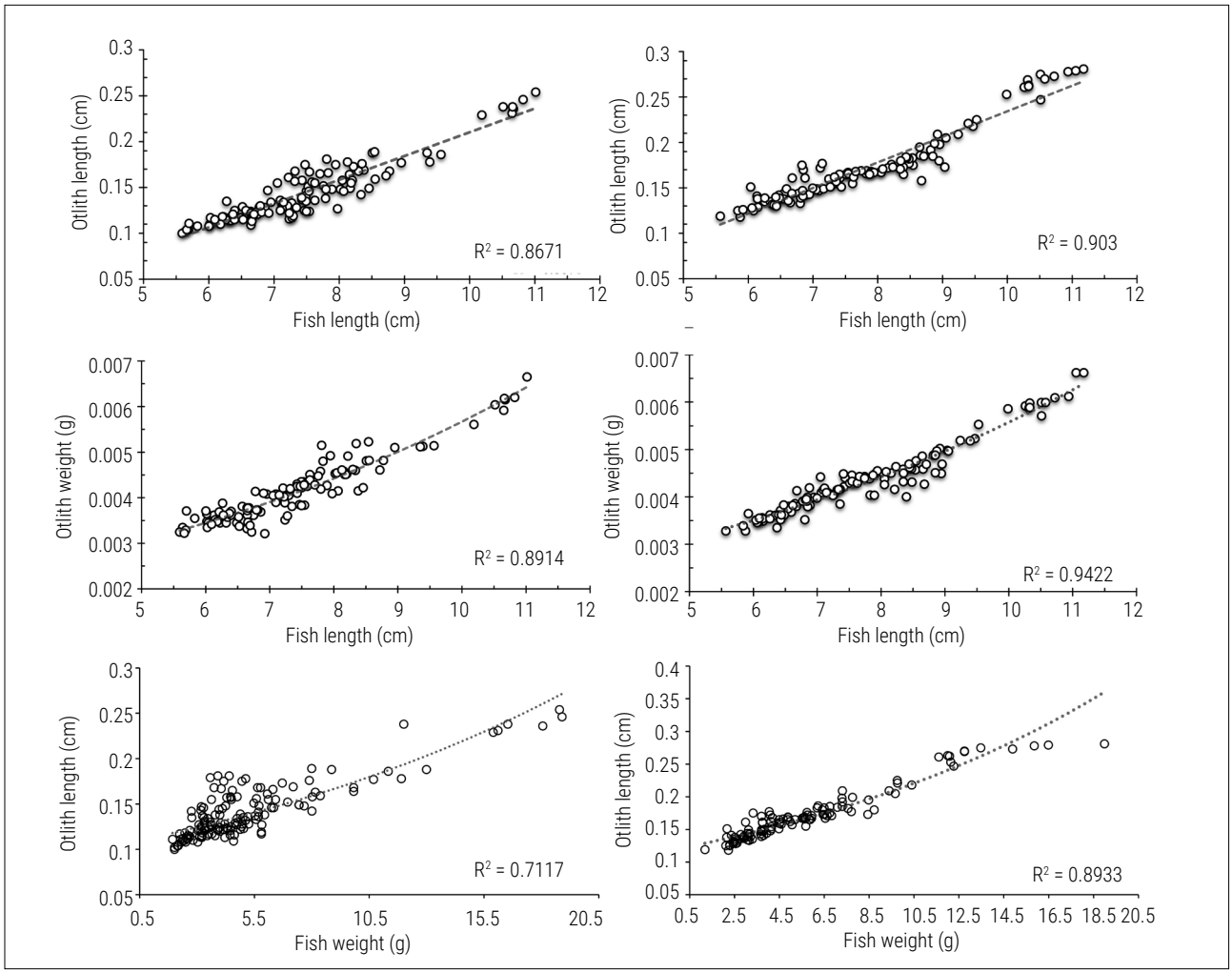

Fig. 6. Fish and otolith morphometric regression plot for the (a) Ganga and (b) Punpun rivers of Bihar, India

(Froese and Pauly, 2019). Rahman *et al.* (2018) and Ahirwal *et al.* (2022) reported the maximum length of 11.34 and 11.51 cm from Bangladesh and India, respectively which are in contrast to present study. In fisheries management, information about morphological parameters are very important and can be used to estimate various growth and population parameters. The morphological parameters of a fish can vary from place-to-place owing to its different environmental parameters, food resources, region specific gear selectivity, and market preferences (Hossain *et al.,* 2012; Ahirwal *et al.,* 2022).

The value of slope *b* in the regression model is very important for the length-weight relationship study, and the value of *b* for most of the species lies between 2.5 and 3.5 (Froese, 2006). The LWR could help in the prediction of the health status of fish stocks in a particular ecosystem. In the present study, the overall value of *b* was >3, indicating positive allometric growth for both the river ecosystems, where width increases faster than length of species, and this happens when species are in good condition (*i.e.*, temperature, food availability and environmental condition). Similar to the present findings, isometric to positive allometric growth of this species from the Ganga River and wetlands of Assam and West Bengal in India has been reported by various authors (Mitra *et al.,* 2005; Pal *et al.* 2013; Kaushik and Bordoloi, 2015; Ahirwal *et al.,* 2022). Similarly, Hossain *et al.* (2018) also observed isometric to positive allometric growth of *P. sophore* in river, pond, and beel ecosystems of Bangladesh. Conversely, negative allometry (male *b* =1.92; female *b* = 2.07) was obtained from the tanks of Karnataka, India (Kiran, 2015). The more difference in the value of *b*, the greater the chances of changing conditions *i.e.*, health, environmental, life stage and diet composition (Ahirwal *et al.,* 2018; Gogoi *et al.,* 2021).

Different condition factors (K_A Allometric; K_F Fulton's, and Kn Relative) were analysed to evaluate the condition of fish health and habitat status. In the present investigation, the mean value of K_F from both the rivers was found to be >1), which specifies that the species thrives well in both the ecosystems. Likewise, Hossain *et al.* (2018) and

Ahirwal *et al.* (2022) reported K_F values of 1.14 and 1.15 from Padma and Ganga rivers in Bangladesh and India respectively. Among these condition factors, Fulton's condition factor K_F was statistically significant ($p<0.05$) for both the river samples, while the relative condition factor (Kn) was significant only for the Punpun River samples. Therefore, Fulton's condition factor was found to be the most accurate method for evaluating the health of *P. sophore* in various ecosystems. Hossain *et al.* (2018) demonstrated that Fulton's condition factor is useful for analysing the condition of *P. sophore*.

Fish morphometrics and otolith relationship

The fish otolith attribute is not only useful for species identification, but also for determining the morphology, age, stock structure and habitat condition based on otolith morphometric information (Seshappa, 1999; Kumar *et al.*, 2017; Pradhan *et al.*, 2022). The current study found a strong correlation between fish length-weight and otolith morphometrics, indicating that otoliths grow alike fish growth. In most studies, the relationship between fish and otolith morphological parameters was evaluated using a linear regression model (Battaglia *et al.*, 2015). However, the exponential regression model resulted in the highest R^2 value when analysing the relationship between fish otolith and fish length. As compared to the Ganga River samples, higher R^2 values were obtained from the Punpun River samples. The result of otolith weight and fish length showed a stronger correlation compared to fish length and otolith length. Thus, the wellbeing of *P. sophore* can be effectively predicted when information on otolith weight is available. Similar types of assertions are depicted in earlier research (Jawad *et al.,* 2011; Kumar *et al.*, 2017; Rani *et al.,* 2019; Chanthran *et al.*, 2021; Pradhan *et al.*, 2022).

The size of a fish otoliths varies with the fish age and development based on the amount of $CaCo_3$ deposited in the sagitta and other biological characteristics (Kumar *et al.*, 2012). According to Li *et al.* (2010), the structure of otoliths can change based on location and biological parameters. Chanthran *et al.* (2021) stated that younger age group of fishes has a strong correlation between fish and otolith morphometrics compared to the older age group. This is because the deposition is uniform during juvenile stage, but shifts to the anterior-posterior axis as the otolith grows with the fish, resulting in otolith elongation.

According to morphometric analysis, otolith dimensions (length, width, and weight) provide valid estimates of fish body size for all species, and are one of the most straightforward ways to obtain information on the fish population and stock structure within an ecosystem and the prey-predator relationship of carnivore (Piscivores) species (Yilmaz *et al.*, 2015; Kumar *et al.*, 2017; Chanthran *et al.,* 2021; Pradhan *et al.,* 2022). The multiparametric findings of the present study revealed that *P. sophore* thrives under suitable ecological conditions in both the riverine ecosystems studied. However, fish length and weight frequency distribution for the Punpun River showed more instances of distribution than for the Ganga River system, and the 'condition factor' suggests that both riverine systems offer healthier ecosystems. However, the maximum length observed for the species earlier was not recorded during this study. This could be because of the way the fish samples were caught and the mesh size of the particular gear, or it could be an alarming sign that needs to be addressed in the future by reducing overfishing, regulating mesh size, and protecting the species by not allowing fishing during its breeding season. The results of this study provide valuable information regarding the relationships between the length, weight, otolith length, otolith breadth, and otolith mass of *P. sophore*.

This forms the first report on the relationships between length, weight, and otolith dimensions of *P. sophore* from the Ganga and Punpun rivers in India. LWRs and condition factor illustrates good ecological conditions for *P. sophore* in the Ganga and Punpun rivers in India. These relationships are also significant to the fisheries sector as they aid in determining the optimal length and time to harvest this species. The data will be useful for future studies comparing the geo-locations and time series data. Growth and otolith morphometric connections for this species suggest that all otolith measurements can be used to estimate the fish size. Changes in the surrounding environment, seasonal fluctuations, the timing of samples, or a combination of these factors could have contributed to the observed disparities in current studies. Therefore, more thorough studies need to be conducted, standardising sample size, sampling depths, as well as sampling season, incorporating studies on the biology of fish, prior to deriving further conclusions.

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