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## Stock structure of small indigenous and near threatened *Ailia coila* (Hamilton, 1822) from Ganga and Brahmaputra river systems

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### Abstract

*Ailia coila* (Hamilton, 1822) commonly called as Gangetic ailia is a “Near Threatened” species in the IUCN Red list. Total of 421 samples were taken from the Ganga (Samudragarh and Farakka) and Brahmaputra (Dhubri and Guwahati) river systems for the present study. The landmark-based truss network system was employed to delineate the stock of this species. The truss network was constructed by using 12 landmarks and 25 truss distances were extracted. Factor analysis using varimax rotation for differentiating the population explained 74.53% of the total variation in the data from first three factors; with first, second and third factors contributing 56.32%, 10.83%, 7.38% of the variations respectively. The morphological difference between the stocks of this species was related to oblique depth measurement on the dorsal and anal fin, and also on the caudal peduncle region. Discriminant function analysis was employed to reveal the percentage correctly classified individuals on the respective locations. Overall classification rate is found to be good around 97.85%. The percentage correctly classified was highest in Samudragarh (100%) which was followed by Farakka, Dhubri and Guwahati. The results from this present study reveals the existence of difference stock in Ganga and Brahmaputra and it forms a baseline study for the stock-specific management measures. Further, genetic population structure of this species should be studied using molecular markers to validate the results.

**Keywords:** Stock, gangetic ailia, truss network analysis

### Introduction

The term ‘Stock’ is a sub-set of species having similar growth and mortality parameters and inhabits in a particular geographical location<sup>[8]</sup>. It is a fundamental concept for both fisheries and endangered species management<sup>[4]</sup>. Knowledge on stock structure, fish biology and stock assessment are prerequisite for formulating an appropriate management policy for a fishery which eventually plays a vital role in safeguarding the existing resources<sup>[5, 6]</sup>. Use of stock concept studies to determine the reproductive resistance to exploitation, is common to a population or individual of subpopulations<sup>[11]</sup>. The morphometrics is the field that deals with the ways of description and statistical analysis of shape variation within and among samples of organisms and the analysis of shape alteration as a result of growth, experimental treatment and evolution<sup>[23]</sup>. Morphometric variations have been recognized as a powerful and essential basis for evaluating the population structure and as a basis for identifying stocks which may be more applicable for studying short-term, environmentally induced variation; perhaps more applicable for fisheries management<sup>[10, 5]</sup>.

The truss network system<sup>[24]</sup> is the landmark-based technique of geometric morphometrics, which poses no restriction on the directions of variation and localization of shape changes, and is much useful in capturing information about the entire fish in a uniform network, and theoretically it should increase the likelihood of extracting morphometric differences between specimen<sup>[27]</sup>. Truss network application has brought greater insight into the biological interpretation of size and shape differences than traditionally used methods.

*Ailia coila* (Hamilton, 1822), commonly called as Gangetic ailia, is native to Asian regions of Pakistan, India, Bangladesh and Nepal and found in large and connected rivers in shoals, taking food from surface and middle layers of the river<sup>[21]</sup>. It forms an important local and artisanal fishery in Ganga and Brahmaputra river systems.

It can grow upto a maximum total length of 30 cm. Throughout its distribution, this species is relatively abundant, and there is no empirical data available regarding the decline in catch in its entire range except in studies of Patra *et al.* (2005) [20] and Mishra *et al.* (2009) [19], who have observed decline in population by 30-80% in different parts of West Bengal. Nevertheless, the fish is caught commonly throughout its range. Overexploitation is one of the major reasons attributed to decline in this population, thereby, rendering it as a “Near Threatened” species in IUCN Red list [13]. The captive breeding of this species is not yet standardized, so the market demand for this species is met only through the wild populations from the rivers. Therefore, effective management of the resource is essential, in order to avoid further depletion of the resources.

## Materials and Methods

### Sampling

*A coila* was identified as per the description of Talwar and Jhingran (1991) [26]. A total of 421 samples, without physical damages, were collected from 4 locations; Samudragarh and Farakka fish market in Ganga and, Dhubri and Guwahati in the Brahmaputra between September 2016 to March 2017 (Fig 1)

### Digitization of samples

Digitization of the image was done immediately after collecting the individuals from the landing centers. The samples were first cleaned in running water, wiped with cotton and placed in the flat platform with vertical and horizontal grids (Plate 1). The fins were erected and placed on the platform in such position that it makes the origin and insertion point visible. Each was labeled with a specific code to identify it in the image. For digitizing the image of individual fishes, Nikon Coolpix L840 16.0 MP Digital Camera was used. The camera was mounted on a leveling tripod with bubble level as an indicator of inclination. The inclination of tripod and platform were leveled by the bubble level for perfect alignment. After digitization of images, each specimen was wrapped in an aluminium foil, labelled and preserved in the freezer at - 20° Celsius.

### Truss morphometric measurements

The truss protocol of *A. coila* in the present study was based on twelve landmarks (Table 1). A truss network was constructed by interconnecting the twelve landmarks to form a total of 25 truss points for a total of 421 numbers of specimens collected. (Table 2 and Plate 2). The extraction of truss distances from the digital images of specimens was conducted using a linear combination of two software platforms, tpsDig2 v2.1 [22] and Paleontological Statistics (PAST) [9]. The truss protocol of *A. coila* in the present study was based on twelve landmarks. There was a significant correlation among all the extracted truss distances and standard length of fish. In order to overcome the size-dependent variations resulted from the allometric growth of fish, the absolute measurements are transformed into size independent variables using the formula [7].

$$D_{trans} = D \times (SL_{mean}/SL)^b$$

Where,

D = original truss measurements / original morphometric measurement

SL = standard length of fish

SL mean = overall mean of standard length

b = within group slope of the geometric mean regression calculated with log-transformed variables, D and SL.

### Analysis of Data

To analyze the significant differences between the locations Multivariate Analysis of Variance was employed. The 25 truss measurements were subjected to Principal Component Analysis which were used to extract the factors. Only retained factors were subjected for rotation procedure by Varimax rotation. The factor scores more than 0.50 was considered to be highly significant [18]. Hence, in this study only factors which are above 0.70 was considered for further analysis. Discriminant Function analysis was typically used to predict the spatial differences between the stocks [16]. It was done by taking the selected factors from factor analysis. Jack Knifed classification is known as leave out classification which involves removing an observation from the data and classifying the observed values based on the remaining data [15]. Khan *et al.* (2013) [17] stated that the number of misclassified individuals in a stock reveals the degree of intermingling among the populations [17]. All the statistical analysis has been done in STATISTICA 8.

### Results

All the size transformed truss measurements were subjected to a Principal component analysis (PCA) using varimax rotation for differentiating the population. The truss distances having factor loadings > 0.80 were considered to be significant. The first three factors of PCA explained 74.53% of the total variation in the data; with first, second and third factors contributed 56.32%, 10.83%, 7.38% of the variations respectively (Table 3).

The variables T3-T4, T4-T9, T4-T10, T5-T9, and T8-T9 had the highest loading on the Factor-1. These factors were concentrated on middle portions of the fish which includes oblique and lateral depth measurements (Fig. 2a). The variables T4-T5 and T4-T8 loaded on Factor-2 were related to dorsal and anal fin regions (Fig 2b). Factor 3 was loaded heavily with truss variables T5-T7, T6-T8 and T7-T8 which were related to the region between the posterior edge of the anal fin and caudal fin region (Fig. 2c).

Location wise bivariate plot of scores of Factor-1 and Factor-2 showed four different populations in Samudragarh, Farakka, Dhubri and Guwahati (Fig. 3)

The variables with high factor loadings were taken for discriminant function analysis. The cross validated classification of discriminant function analysis showed 97.85% correctly classified individuals in their respective locations. The percentage correctly classified was highest in Samudragarh (100%) which was followed by Farakka (97.44), Dhubri (96.25) and Guwahati (97.56) (Table 4).

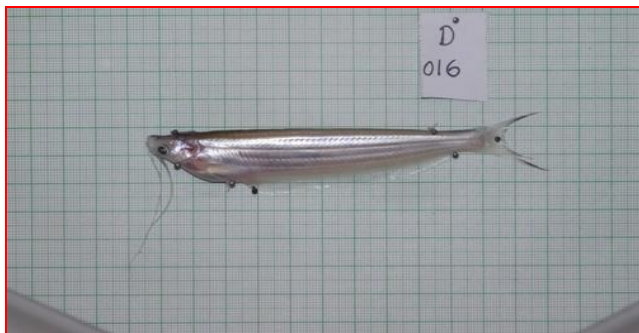
### Discussion

Stock identification of a species has commonly relied on description of unique sets of morphological characters. The formation of biologically meaningful grouping of species in a particular area was the primary mode to retain the Principal components in PCA. Thus with the help of rotated factors the traits which contributed significantly to the factors are identified.

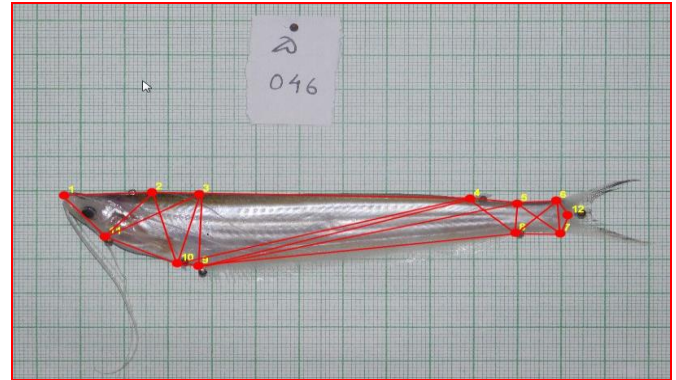
The landmark-based truss network analysis indicated the significant difference between the morphological characteristics of the stocks of *A. coila* from the two river systems. The first three factors explained 74.53% of the total

variation in the data and careful analysis of the factors suggested that the traits associated with shape belonging to middle portion of the body were loaded on the first factor, the shape belonging to the dorsal fin region and anal fin region were loaded on the second factor and the shape belonging to the anal fin region and caudal fin region were loaded on the third factor. Among the three factors, the middle portion of the body of the specimen showed more significant variation. The variation in the middle portion of the body may be due to the more gut content and reproductive organs which corresponded to variation in stock. The variation in the portion between the dorsal fin and anal fin may be due to the different current pattern of the Ganga and Brahmaputra rivers. The fishes are very sensitive to environmental parameters and tends to show high plasticity in morphological characters due to the differences in food, temperature etc. [25, 2, 3]. According to Abdurahman *et al.* (2016) [1] clear separations of all the species in Plicofollis group based on truss network analysis was represented by body depth and caudal regions of the fish. Tzeng (2004) [28] observed two currents (Kuroshio and China coastal current) had a significant effect on the separation of the stocks of spotted mackerel off Taiwan. Jaysankar *et al.* (2004) [14] observed through the principal component analysis of truss landmark variables of Indian mackerel, the area encompassing depth between the origin of anal and origin of second dorsal and caudal peduncle depth to be high loading component. The variation in caudal fin region and anal fin region in *A. coila* from Ganga and Brahmaputra rivers could be a consequence of phenotypic plasticity in response to hydrological conditions. Imre *et al.* (2002) [12] observed deeper caudal peduncle from high turbulent water in brook charr which may be true in the present study also. Verma and Serajuddin (2016) [29] revealed three sub population of *Eutropiichthys vacha* with the classification rate of 91.7% from Discriminant function analysis.

The bivariate plot of rotated Factor-1 against Factor-2 showed separation of stocks vertically and horizontally. The factor- 1 and the factor-2 plot shows clear separation of Guwahati and Samudragarh stocks on the vertical axis and separation of Farakka and Dhubri stock with slight mixing on the horizontal axis. Therefore, from present study it is appears that the four stocks may be entirely different from each other, however, it is suggested that the results should be validated through molecular methods before used for management purposes.



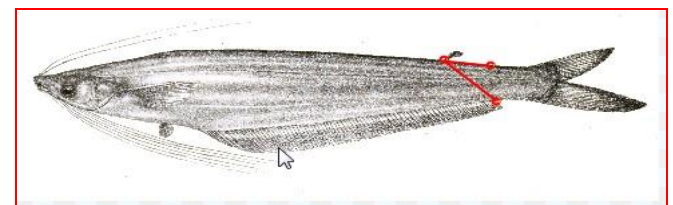
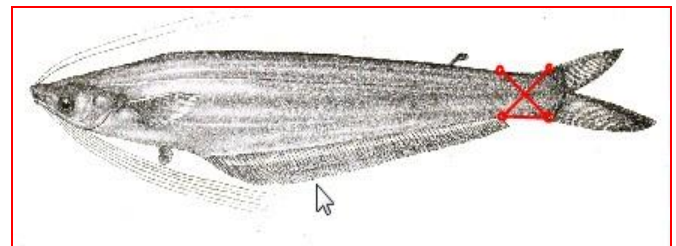
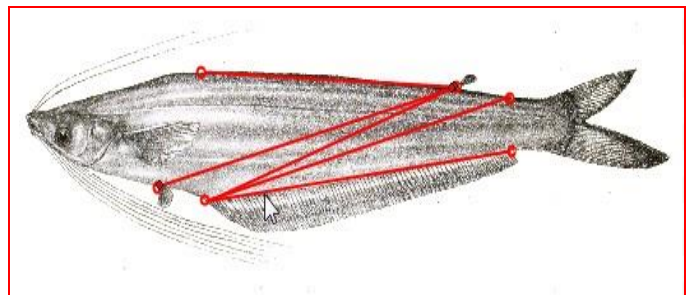
**Plate 1:** *A. coila* on flat platform with vertical and horizontal grids labelled with a specific code



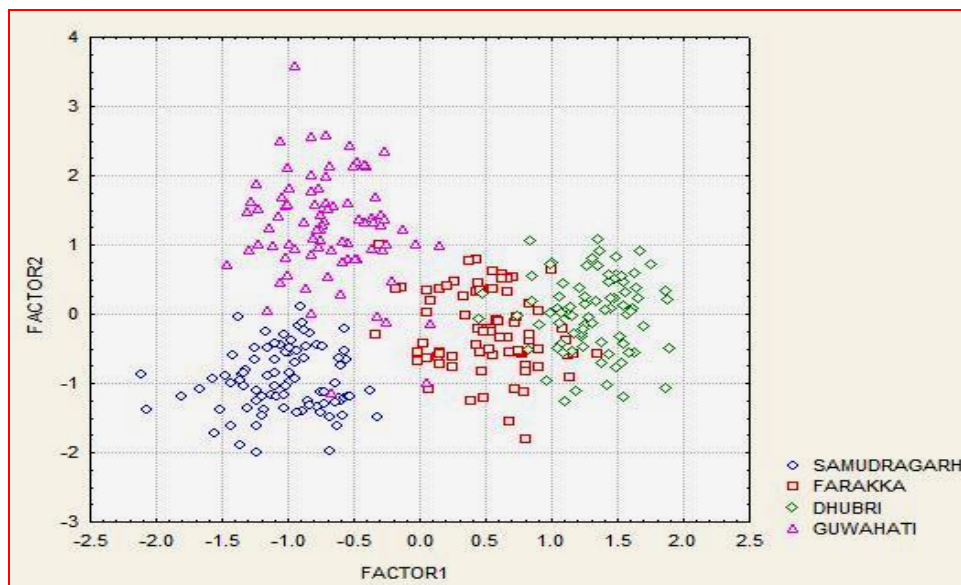
**Plate 2:** Truss network of *A. coila* showing the variables interconnecting 12 landmarks



**Fig 1:** Details of different sampling location



**Fig 2a, 2b, 2c:** Distances with meaningful loadings on first 3 factors in truss network analysis of *A. coila*



**Fig 3:** Location wise bivariate plot of scores of the two factors (Factor-1 and Factor-2) extracted from Truss data of *A. coila*

**Table 1:** Landmarks used for the extraction of truss measurements from *A. coila*

Landmark number	Landmark position
01	Anterior tip of snout on upper jaw
02	Posterior edge of supra occipital bone
03	Exactly opposite to origin of anal fin on dorsal side
04	Origin of dorsal adipose fin
05	Exactly opposite to posterior edge of anal fin on dorsal side
06	Dorsal origin of caudal fin
07	Ventral origin of caudal fin
08	Posterior edge of anal fin
09	Origin of anal fin
10	Origin of pelvic fin
11	Intersection of operculum and ventral contour of body
12	Standard length

**Table 2:** Distances obtained from the selected twelve landmarks

SL No.	Truss distance	Description
01	t1-t2	Distance between the tip of the snout and posterior edge of supra occipital bone
02	t1-t11	Distance between the tip of the snout and intersection of operculum and ventral contour of body
03	t2-t3	Distance between posterior edge of supra occipital bone and exact opposite of origin of anal fin on dorsal side
04	t2-t10	Distance between posterior edge of supra occipital bone and origin of pelvic fin
05	t2-t11	Distance between posterior edge of supra occipital bone and intersection of operculum and ventral contour of body
06	t3-t4	Distance between exact opposite of origin of anal fin on dorsal side and origin of dorsal adipose fin
07	t3-t9	Distance between exact opposite of origin of anal fin on dorsal side and origin of anal fin
08	t3-t10	Distance between exact opposite of origin of anal fin on dorsal side and origin of pelvic fin
09	t3-t11	Distance between exact opposite of origin of anal fin on dorsal side and intersection of operculum and ventral contour of body
10	t4-t5	Distance between origin of dorsal adipose fin and exact opposite of posterior edge of anal fin on dorsal side
11	t4-t8	Distance between origin of dorsal adipose fin and posterior edge of anal fin
12	t4-t9	Distance between origin of dorsal adipose fin and origin of anal fin
13	t4-t10	Distance between origin of dorsal adipose fin and origin of pelvic fin
14	t5-t6	Distance between exact opposite of posterior edge of anal fin on dorsal side and dorsal origin of caudal fin
15	t5-t7	Distance between exact opposite of posterior edge of anal fin on dorsal side and ventral origin of caudal fin
16	t5-t8	Distance between exact opposite of posterior edge of anal fin on dorsal side and posterior edge of anal fin
17	t5-t9	Distance between exact opposite of posterior edge of anal fin on dorsal side and origin of anal fin
18	t6-t7	Distance between dorsal origin of caudal fin and ventral origin of caudal fin
19	t6-t8	Distance between dorsal origin of caudal fin and posterior edge of anal fin
20	t7-t8	Distance between ventral origin of caudal fin and posterior edge of anal fin
21	t8-t9	Distance between posterior edge of anal fin and origin of anal fin
22	t9-t10	Distance between origin of anal fin and origin of pelvic fin
23	t10-t11	Distance between origin of pelvic fin and intersection of operculum and ventral contour of body

**Table 3:** Variable loading for the truss data from rotated factor

Truss distance	Factor – 1	Factor - 2	Factor – 3
T1T2	0.164009	0.604963	0.301297
T1T11	0.169599	0.522283	0.262640
T2T3	0.279291	0.456091	0.387510
T2T10	0.493419	0.641649	0.293214
T2T11	0.256358	0.708205	0.330477
T3T4	0.961367	0.063883	0.228377
T3T9	0.332131	0.591927	0.480150
T3T10	0.241154	0.600964	0.482484
T3T11	0.369861	0.680561	0.466425
T4T5	0.138501	0.884582	-0.077797
T4T8	0.183983	0.892454	0.221831
T4T9	0.953163	0.114910	0.257495
T4T10	0.921335	0.115495	0.321025
T5T6	0.369865	0.077778	0.798920
T5T7	0.422121	0.272389	0.805
T5T8	0.233775	0.484475	0.631706
T5T9	0.911332	0.334796	0.204123
T6T7	0.505974	0.485923	0.506970
T6T8	0.387098	0.287435	0.807926
T7T8	0.327900	0.113282	0.806572
T8T9	0.919797	0.315267	0.194538
T9T10	0.124531	0.059898	0.467550
T10T11	0.580645	0.547554	0.195388

**Table 4:** Cross validated classification matrix of truss data – location wise

Location	Percent – correct (%)	Samudragarh	Farakka	Dhubri	Guwahati
Samudragarh	100.	87	0	0	0
Farakka	97.43	0	76	2	0
Dhubri	96.25	0	3	77	0
Guwahati	97.56	0	2	0	80
Total	97.85	87	81	79	80

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