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Effect of formalin preservation on morphology and condition factor of the freshwater shark *Wallago attu* (Bloch and Schneider, 1801) using truss network system

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ABSTRACT

The study was aimed to evaluate the effect of formalin preservation on the shape, size and condition factor of the freshwater silurid catfish *Wallago attu* (Bloch and Schneider, 1801) using length-weight relationship (LWR), condition factor and truss analysis. A total of 50 specimens of the fish were procured from the river Ganga at Kanpur region. The fresh and formalin preserved fish were measured for total length, standard length and body weight. Results of LWR revealed that the fresh and preserved fish followed different growth pattern and cube law. High values of r^2 were obtained for the relationship between length and weight of the fish before and after preservation of the fish in formalin. Decrease in length and an increase in weight was observed in the preserved specimens of the fish due to the effect of formalin but were not statistically significant. The mean condition factor (K) showed significant ($p < 0.05$) change in the fish after preservation. The univariate and multivariate analysis of truss measurements, two partially overlapping different convex hulls of the scatter plot of PC1 on PC2 and canonical discriminant functions showed highly significant morphometric heterogeneity between the specimens of fresh and preserved fish. The results of the present study suggest that fresh specimens of the fish should be used in research studies based on weight and linear body measurements of the fish.

Keywords: Condition Factor, Formalin, Length-weight relationship, Truss network system

Formalin is widely used to preserve fish specimens for their future use in taxonomic, biosystematic and biological studies. There are reports that formalin can influence the colour, texture, length, weight and the body shape of the fish after their preservation for longer period of time (Ajah and Nunoo, 2003; Vervust *et al.*, 2009; Martinez *et al.*, 2013). Al-Hassan *et al.* (2000) reported that the preservatives such as formalin and alcohol affect the morphometric characters such as head length (HL), standard length (SL) and total length (TL) in two species of mullidae. The equation of length-weight relationship (LWR) is widely applied in fisheries and conservation studies to calculate the fish biomass at a given length (Sinovci *et al.*, 2004; Froese, 2006) which is also used for the translation of the growth-in-length equation to growth-in-weight of the fish (Wootton, 1990; Moutopoulos and Stergiou, 2002). Condition factor is an indicator of the health and well-being of individual fish in their habitat (Nehemia *et al.*, 2012; Stavrescu-Bedivan *et al.*, 2015). An image of entire body shape of the fish is required to evaluate the intra/inter specific morphometric differences in fish under truss analysis (Turan, 2006) which is considered to be helpful for the measurement of shape variation. The freshwater fish, *Wallago attu*

(Bloch and Schneider, 1801) is an economically important inland water teleostean fish of the family Siluridae, native to South and South-east Asia and commonly known as freshwater shark (Talwar and Jhingran, 1991; Giri *et al.*, 2002; Mirza, 2003). A survey of literature showed that no information on the effect of formalin preservation on morphology of *W. attu* is available. Therefore, the present study was planned to investigate the effect of formalin preservative on the length, weight measurements and the shape of the fish so that the influence of formalin on LWR, length-length relationship (LLR), morphology and condition factor of fish can be assessed. Efforts were made to know whether formalin preservative affect linear measurements of the fish.

A total of 50 samples of *W. attu* were procured randomly between October 2018 to January 2019 from the river Ganga (30°55'N; 70°7'E) at Kanpur region using cast and drag nets with the help of local fisherman. The fresh specimens were measured for lengths (total and standard length) in cm, weight in g respectively and preserved in 10% formalin for a period of one month. The length and weight measurements, condition factor, colour and shape of the fish was compared between fresh and preserved specimens using truss network system.

The LWR and LLR equations were derived using the least square formula as suggested by Ricker (1973):

$$W = aL^b$$

where, 'W' is body weight in g, 'L' is total length in cm, 'a' is the intercept of regression line and 'b' the slope of regression line.

The condition factor (K) was calculated using the equation:

$$K = W \times 100/L^3$$

where 'K' is Fluton's condition factor and the factor 100 is used to bring K close to unity.

Coefficient of determination (r^2) was calculated in order to indicate the quality of linear regression. Paired t-test was performed to compare the size difference, condition factor and the value of ' r^2 ' of different equations of LWR and LLR of the specimens of fresh and preserved fish.

For morphometric study, fresh and preserved specimens were placed on a truss paper and images were taken out using digital camera. Landmarks were digitised in software, TpsDig version 1.40 (Rholff, 2004). A total of 8 homologous landmarks were taken for each fish sample and the truss network system was constructed by joining 8 landmarks to form 16 truss distance measurements (Fig. 1b-d). The size effect of the samples of the data was removed using the formula as given by Elliott (1995):

$$M_{adj} = M \cdot (L_s/L_o)^b$$

where M is original measurement, M_{adj} is size-adjusted measurement, L_o is standard length, L_s is the mean of standard length and parameter b was estimated for each character from observed data as the slope of regression of $\log M$ on $\log L_o$.

After data transformation, all the truss measurements were subjected to one-way ANOVA to find out the significant ($p < 0.05$) differences in truss measurements which were further subjected to principal component analysis (PCA) and discriminant function analysis (DFA) to elucidate the variation in shape of fresh and preserved specimens of the fish. All the calculations were done with the help of Graph Pad Prism 5, PAST (v 3.0) and SPSS (v 12.0) softwares.

Details of the equations of LWR, LLR, 95% confidence level of 'a' and 'b' and values of coefficient of determination (r^2) of $W. attu$ are given in Table 1. Change in body colour was observed after preservation in 10% formalin (Fig. 1a and c). Relationship between TL-BW of the fresh and preserved fish showed isometric ($b=3.024$) and allometric ($b=2.627$) growth pattern respectively (Fig. 2). Linear regression lines were obtained for relationships between TL-BW and TL-SL in the specimens of both fresh and preserved fish (Fig. 3). Decrease in length and increase in weight of the fish was observed after the preservation due to the effect of formalin but the paired t-test analysis revealed that the mean length and weight of the fresh fish was not significantly different ($p < 0.05$). The difference in the values of mean condition factor before (0.466) and after (0.493) preservation was also statistically significant ($p > 0.05$).

All the truss measurements were found to be highly significant ($p < 0.001$) in one-way ANOVA and were further tested by multivariate analysis using PCA and DFA where 27 principal components were extracted. Total variance of PC1, PC2 and PC3 showed a value of 44.81, 24.15 and 15.48% respectively (Table 2). Two different convex hulls were obtained in scatter graph of PC1 on PC2 (Fig. 3) which indicated a significant variation between body shapes of fresh and preserved specimens. In DFA,



Fig. 1. Colour and location of 8 morphometric landmarks for constructing the truss network system on *W. attu*. a and c: Before preservation; b and d: After preservation

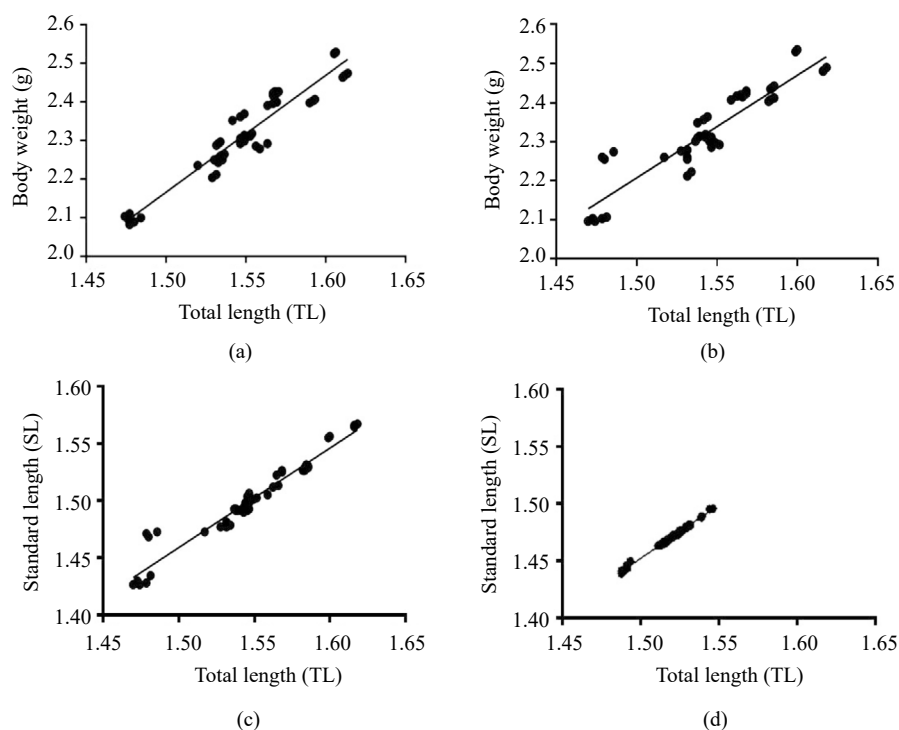


Fig. 2. Length-weight and length-length relationship in *W. attu*. a and c: Fresh fish; b and d: Preserved fish

Table 1. Regression parameters, correlation and condition factor of *W. attu* before and after formalin preservation

Logarithmic regression equation	Before preservation		After preservation		Significance level 0.05 for r^2	p - value
	TL vs BW	TL vs SL	TL vs BW	TL vs SL		
	-2.369 + 3.024	-0.034 + 0.989	-1.732 + 2.127	0.152 + 0.870	Yes	0.0001
' r^2 '	0.893	0.976	0.842	0.931	Yes	0.003
'K' value	0.466		0.493		Yes	0.017
95% CL of 'a'	-2.840 to -1.898	-0.103 to 0.034	-2.241 to -1.224	0.046 to 0.258		
95% CL of 'b'	2.720 to 3.328	0.945 to 1.034	2.298 to 2.955	0.802 to 0.939		

TL=Total length, SL=Standard length, BW=Body weight, 'a'= Intercept, 'b'=Slope, CL= Confidence level, r^2 =Coefficient of determination, 'p'= Significance level and K=Condition factor

91.3% of original grouped cases were correctly classified into one discriminant function (DF1). Assessment of the pooled within-group correlations of discriminating functions and morphometric measurements showed that some measurements exhibited differences between the samples (Table 3). Separate group plots of fresh and preserved specimens indicated no overlapping, but a significant variation (Fig. 4).

The rate of increase in weight in relation to length (slope of regression) in the present study was significantly lower ($p < 0.05$) in the preserved fish ($b = 2.627$, allometric growth) compared to that of fresh fish ($b = 3.024$, isometric growth) because of increase and decrease of weight and length respectively during preservation in 10% formalin.

The present findings revealed that the body length in *W. attu* decreased due to the folding and shrinkage of the body after preservation in 10% formalin. Our results are in agreement with the previous studies of Shields and Carlson (1996); Ajah and Nunoo (2003); as well as Greszkiewicz and Fey (2018).

The value of coefficient of determination (r^2) between length-weight and length-length relationship in both fresh and preserved fish was high indicating a strong relationship, but there was a significant difference in the value of r^2 in both LWR and LLR before and after preservation of the fish ($p < 0.05$) suggesting the use of fresh samples for morphometric studies. Similarly, Kruse and Dalley (1990) also suggested the use of fresh specimens

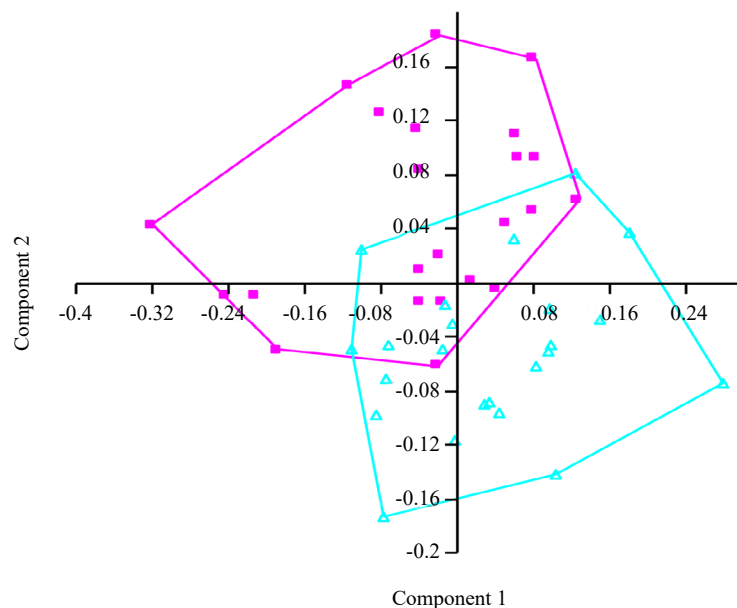


Fig. 3. Scatter plot of PC1 on PC2 showing convex hulls

Table 2. The first three principal components extricated from principal component analysis

S. No.	Morphometric measurements	PC1	PC2	PC 3
1	1-2	0.204	-0.799	0.443
2	1-3	-0.103	0.332	0.513
3	1-5	-0.262	-0.023	-0.043
4	1-6	-0.678	-0.034	0.050
5	1-7	-0.002	-0.082	0.429
6	1-8	0.189	-0.563	0.406
7	2-3	-0.261	0.927	-0.102
8	2-4	-0.192	0.782	-0.499
9	2-5	-0.275	0.712	-0.477
10	2-6	-0.483	0.673	-0.415
11	2-7	-0.224	0.664	-0.036
12	2-8	0.121	-0.600	0.469
13	3-4	0.120	-0.229	-0.622
14	3-5	-0.035	-0.182	-0.616
15	3-6	-0.363	-0.195	-0.549
16	3-7	-0.129	0.071	0.100
17	3-8	-0.234	0.463	0.460
18	4-5	0.296	0.457	0.808
19	4-6	0.562	0.336	0.635
20	4-7	-0.101	0.013	0.156
21	4-8	-0.210	0.319	0.102
22	5-6	0.981	0.075	-0.167
23	5-7	-0.216	-0.030	0.001
24	5-8	-0.346	0.258	-0.020
25	6-7	-0.421	-0.025	0.055
26	6-8	-0.569	0.226	0.037
27	7-8	-0.139	0.295	0.123
Total variance (%)		44.81	24.15	15.48
Commulative variance (%)		44.81	68.96	84.44

Table 3. Pooled within-groups correlations between discriminating morphometric measurements and standardised canonical discriminant functions. Variables ordered by absolute size of correlation within function

S no.	Morphometric measurements	DF 1
1	3-8	0.40*
2	4-8	0.39*
3	1-2	-0.37*
4	2-4	0.34*
5	6-8	0.32*
6	2-6	0.31*
7	3-5	-0.28*
8	2-7	0.26*
9	2-8	-0.26*
10	2-5	0.24*
11	5-8	0.23*
12	7-8	0.22*
13	3-4	-0.21*
14	1-3	0.18*
15	3-6	-0.17*
16	1-5	-0.16*
17	4-7	0.14*
18	6-7	0.13*
19	3-7	-0.10*
20	4-6	0.10*
21	1-7	-0.62*
22	5-7	0.03*
23	1-6	0.02*
24	2-3	0.53
25	4-5	0.40
26	1-8	-0.35
27	5-6	-0.23

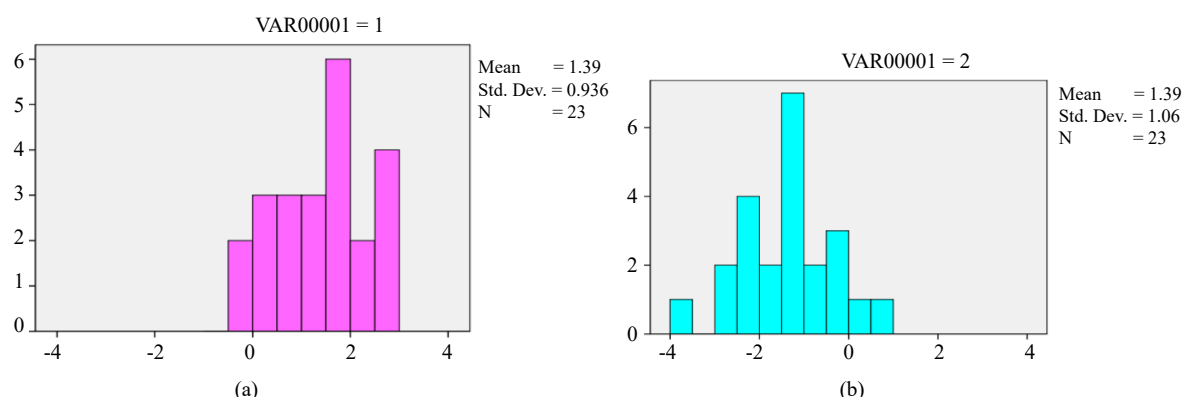


Fig. 4. Separate group plot of *W. attu* (a) Before formalin preservation; (b) After formalin preservation

of fish in order to estimate the correct regression equation for LWR in fishes.

The difference in the values of mean condition factor before and after the fish preservation was also statistically significant ($p < 0.05$) in the present study, as a result of the decrease in length and increase in weight of the fish after preservation in formalin. Ajah and Nunoo (2003) also reported increase in the value of 'K' because of formalin treatment in clupeid fish, *Sardinella aurita* and also emphasised that smoking is not a suitable method for fish preservation if morphometric measurements are to be made, and suggested that fresh samples of fish are the best, followed by freezing and formalin preservation. A significant change ($p < 0.005$) in body shape of the fish was observed between the fresh and preserved samples using truss analysis after preservation in 10% formalin for a period of one month.

A lot of factors such as concentration and kind of chemical fixatives and span of preservation are responsible for various degrees of changes in colour, morphometric and meristic characteristics as well as weight of the fish during preservation (Vajargah and Hedayati, 2014). The chemical and water contents of tissues and the ratio of white to red muscles in the body of fishes are also considered to be important factors which influence the length, weight and colour of the body of the fish during preservation (Leslie and Moore, 1986; Jawad *et al.*, 2001). The change in weight of the fish after preservation could be attributed to the change in water content. Macdonald *et al.* (1997) emphasised that besides age, size, species, developmental state and rigor mortis, the osmoregulatory state of the fish is considered to be important for shrinkage during preservation.

The univariate (one-way ANOVA) and multivariate analysis *via* PCA and DFA showed that there was highly significant ($p < 0.001$) morphometric heterogeneity between

the fresh and preserved samples of the fish. Our study also revealed that fresh specimens of the fish should be utilised for the study on LWR, LLR and condition factor in fish as the length and weight of the fish are affected by the formalin fixative. The colour of fixed sample of *W. attu* also exhibited distinct degree of change.

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