LANDMARK-BASED MORPHOMETRIC ANALYSIS ON BODY SHAPE OF SOME FISH SPECIES IN THAUNG PYIN "IN", THEGON TOWNSHIP, BAGO REGION

Kyawt Kay Khaing[1], Aye Aye Aung[2],

Abstract

The present research focused to examine landmark-based morphometric variations of *Notopterus notopterus*, *Macrognathus zebrinus*, *Anabas testudineus* and *Trichogaster pectoralis* from Thaung Pyin "In", Thegon Township, Bago Region. A total four of 20 specimens of each species were collected from the study area. The 10 morphological landmarks were selected along the outline of the specimens. The number of landmarks based on the respective species and the truss network were also changed on morphology. In the present study 8 to 10 points in landmark and 16 to 21 trust networks were recorded. Multivariate analysis of variance (ANOVA) was carried out to test the significance of morphological differences among the study species. The principal component analysis (PCA) indicated shape variation and explained the total variance (66.03%, 79.44%, 64.78% and 69.66 %) of *N. notopterus*, *M. zebrinus*, *A. testudineus* and *T. pectoralis* respectively. Discriminant function analysis (DFA) was accounted for three morphological indices in *M. zebrinus* , four morphological indices in *N. notopterus* and *A. testudineus,* and six morphological indices in *T. pectoralis* of the within group variability. Individuals into their original group were correctly classified and the result showed 100% in *N. notopterus*, *M. zebrinus* and *T. pectoralis* and 95% in *A. testudineus*. All statistically analyses were carried out using SPSS Software Package Version 25.

Keywords: Landmarks, Truss network, Morphometric variations and Some fishes

Introduction

The estimation of morphological variation within species population could be performed using conventional and truss morphometric approaches (Marcus *et al*,1996). Morphometric is defined as the study of quantitative analysis such as size and shape of living organisms, which for understanding the taxonomy as well (Park, *et. al.,* 2013). Image analysis systems have clearly played an important role in the advancement of morphometric research. Morphometric methods are now more powerful for stock identification through improved data collection, better description of shape, and the potential of new analytical techniques (Walker, 1997). Landmark-based morphometric was carried out to determine the variation of population structure and develop breeding program for sustainable production and conservation (Hossain *et al.*, 2015). The technique using morphometric imposes no restrictions on the direction of variation and localization of shape change and is highly effective in capturing information about the shape of an organism (Cavalcanti *et al*., 1999). Truss network systems constructed with the help of landmark points are powerful tools for stock identification (Turan *et al.,* 2004). Data points were arranged in a "Trusses" around the fish which layout minimize the numbers of measurements and increases the sensitivity of the analysis (Hossain *et al.*, 2010).

The relationships between various body parts of fish can be used to assess the well-being of individuals. Several techniques have been proposed for stock identification, an essential aspect of

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fisheries science management, which focuses on recognizing self-sustaining components within natural populations (Cadrin *et al*., 2004). In Myanmar, numerous studies have examined the taxonomy, distribution, and habitats of freshwater and marine fish across various river systems and floodplains (Day, 1978). Key rivers, such as the Ayeyarwady, Sittaung, and Thanlwin, flow into the coastal zones, supporting diverse aquatic ecosystems (Myint Pe, 2002). Among the fish species in these areas, *Notopterus notopterus* (Pallas, 1769) (Bronze featherback ,locally known as Nga-pe), *Macrognathus zebrinus* (Blyth, 1858)(Zebrafish spiny eel , Nga-mwedo) , *Anabas testudineus*, (Bloch, 1792) (Climbing Perch, Nga-bye-ma) and *Trichogaster pectoralis* Regan, 1910 (Snakeskin gourami , Belar) are significant food sources and play important ecological roles. The study was conducted at Thaung Pyin "In" in Thegon Township, Bago Region, which is fed by several chaungs originating from the Bago Yoma and Pyay Mountain ranges. This research aims to explore and assess their morphometric variations in the same stock. The objectives of this study are to examine and compare the morphometric differences in within species and between species of studied fish.

Materials and Methods

Study Area and Period

The study was conducted at Thaung Pyin “In,” part of the Inma “In” complex in Thegon Township, Bago Region , located around latitude 18∘32′2.26″ N and longitude 95∘21′16.55″ E (Plate 1). A total of 80 individuals (20 for each species) were collected directly from fishermen during December 2023 to August 2024. The collected specimens were immediately transferred to a cold box for transport to the laboratory for identification and morphometric measurements, following the classification systems of Talwar and Jhingran (1991) and Froese and Pauly (2018).

Truss network system

The variation in fish shape and size was assessed using the truss network system. The specimen was laid on the sandwich sheet into right body posture and fins were into a natural position. For example, ten of the morphological landmarks were chosen along the outline of the specimen and pinned down with long different color pins. Then removed specimen, the pinning positions were marked by using the permanent marker pen. Measurements were taken on 21 inter-landmark distances between 10 homologous landmarks using a standard truss network protocol (Fig. 1 and Plate 2) (Strauss and Bookstein, 1982). When the number of landmarks change, the number of truss network measurements known as inter- landmark distances may also change. The identification and classification of the collected fish species were followed after Talwar and Jhingran, 1991 and Froese and Pauly, 2018.

Data analysis

Necessary to remove size-dependent variations from all of the measured morphometric characters, use allometric formula as proposed by Elliott *et al*., 1995.

$M_{adj} = M\,(L_s / L_o)^b$, where M is the original measurement, M_{adj} = The size-adjusted measurement, L_s is the overall mean of the total length samples in each analysis, L_o is the total length and b is the slope of the regression coefficient, was calculated for each character from the observed data as the slope of log M on log L_o. One way ANOVA was used to test the significance of 21 morphological differences. Wilk's lambda (λ) was used to assimilate the variation between and among the entire population. PCA (Principal component analysis) were used decreasing redundancy among the morphometric variables and removing plentiful autonomous variables was carried out Veasey *et al.*, 2001 and Samaee *et al.*, 2006. SPSS (version 25) was used for statistical analyses.

Calculation of Truss measurement

The number of truss measurements was calculated by equation based on landmarks.

If n = 10, truss measurement number = $\frac{5n}{2} - 4$

$= \frac{5\,x\,10}{2} - 4 = 21$ (Strauss & Bookstein, 1928)

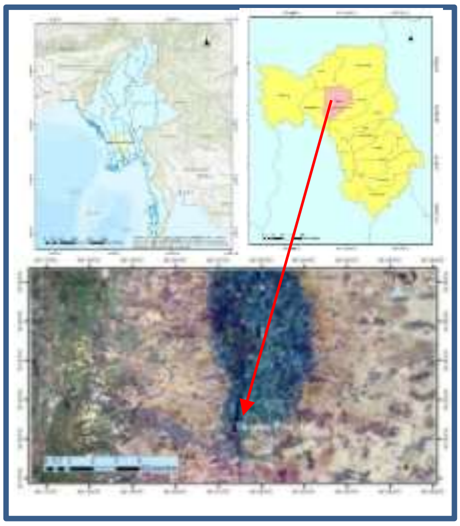


Plate 1 Map of Study Area

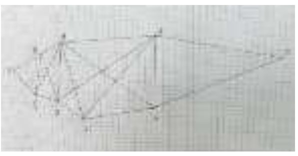
A.*Notopterus notopterus*

B.*Macronagthus zebrinus*

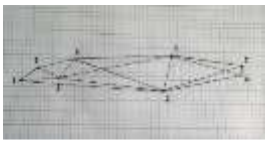
C. *Anabas testudineus*

D. *Trichogaster pectoralis*

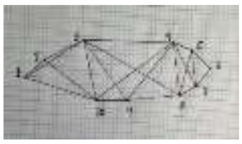
Plate 2 Location of landmarks on the fish



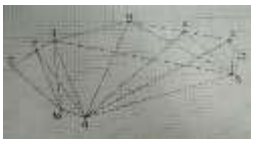
A.*N. notopterus*



B.*M. zebrinus*



C. *A. testudineus*



D. *T. pectoralis*

Figure. 1 Constructing truss network between landmarks

Table 1 Location of landmarks and data sheet for Morphometric measurements

Point	***Notopterus notopterus***	***Macronagthus zebrinus***	***Anabas testudineus / Trichogaster pectoralis***
1	Tip of the snout	Tip of the snout	Tip of the snout
2	Upper end of the operculum	Origin of the eye	Upper end of the operculum/ End of the eye

Point	*Notopterus notopterus*	*Macronagthus zebrinus*	*Anabas testudineus / Trichogaster pectoralis*
3	Beginning of the scaled nape	Origin of the dorsal fin base	Original of the dorsal spine fin / End of the skull
4	Origin of the dorsal fin	End of the dorsal fin base	End of the dorsal spine fin / Origin of the dorsal fin
5	Middle of the caudal fin	Upper origin of the caudal fin	End of the dorsal soft fin/ End of the dorsal fin
6	Opposite of origin of the dorsal fin	Lower origin of the caudal fin	Middle of the caudal fin/ Upper of caudal fin
7	Origin of the pelvic fin	Origin of the anal fin base	End of the anal soft fin /Middle of caudal fin
8	Origin of the pectoral fin	Origin of the pectoral fin base	End of the anal spine fin /Lower of caudal fin
9	End of the maxilla		Origin of the anal spine fin/ Anterior origin of the anal fin
10			Origin of the pelvic fin base/ Anterior origin of the pelvic fin

Results

Four different species of fish, *Notopteurus notopterus*, *Macrognathus zebrinus*, *Anabas testudineus* and *Trichogaster pectoralis* belonging to four families, four genera under three orders were studied. A total of 20 specimens of each species were examined, making 80 specimens in total.

Table 2 Total length of fish species (n= 20)

Sr. No	Species	Total length(mm)		Weight (g)	
		Range	Mean ± SD	Range	Mean ± SD
1	*Notopterus notopterus*	236 – 269	249.30 ±10.04	94-128	109.7 ± 10.01
2	*Macronagthus zebrinus*	130 – 167	149.10 ± 9.58	10-17	13.0 ± 2.03
3	*Anabas testudineus*	114-138	125.35 ± 6.69	26-39	32.05 ± 4.08
4	*Trichogaster pectoralis*	174 – 207	193.55 ± 7.49	77-144	121.1 ± 16.12

The length range and mean with standard deviation for each specimen were shown in table 2. Among the morphometric measurements, one way ANOVA test showed that all truss measurements for two species were highly significant ($P < 0.001$) with Wilk's lambda value $0 < \lambda < 1$. The eigen values of principal component analysis of was *N. notopterus* three factors (6.57 for PC1, 3.47 for PC2 and 1.85 for PC3), *M. zebrinus* was three factors (9.11 for PC1, 2.08 for PC2, 1.52 for PC3), *A. testudineus* (8.44 for PC1, 3.08 for PC2 and 2.09 for PC3) and *T. pectoralis* (10.36 for PC1, 2.41 for PC2 and 1.86 for PC3) in table 3. The principal component analysis (PCA) of 18 morphometric measurements of *N. notopterus* yielded three principal components explaining 66.03 % of the total variance (36.52 % for PC1, 19.26% for PC2 and 10.25 % for PC3), 16 morphometric measurements of *M. zebrinus* yielded three principal components explaining 79.44 % of the total variance (56.96 % for PC1, 12.98% for PC2 and 9.51% for PC3), 21 morphometric measurements of *A. testudineus* yielded three principal components explaining 64.78 % of the total variance (40.17 % for PC1, 14.68% for PC2 and

9.93% for PC3) and 21 morphometric measurements of *T. pectoralis* yielded three principal components explaining 69.66 % of the total variance (49.33 % for PC1, 11.49% for PC2 and 8.84% for PC3)in the entire dataset (Table 3).

Table 3 Eigen-value, percentage of variance and percentage of Cumulative for the study species

Species	Factor	Extraction sums of square		
		Eigen-value	% of variance	Cumulative%
Notopterus notopterus	1	6.57	36.52	36.52
	2	3.47	19.26	55.78
	3	1.85	10.25	66.03
Macrognathus zebrinus	1	9.11	56.96	56.96
	2	2.08	12.98	69.93
	3	1.52	9.51	79.44
Anabas testudineus	1	8.44	40.17	40.17
	2	3.08	14.68	54.85
	3	2.09	9.93	64.78
Trichogaster pectoralis	1	10.36	49.33	49.33
	2	2.41	11.49	60.82
	3	1.86	8.84	69.66

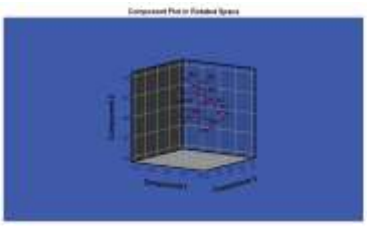
The distance codes of *N. notopterus* are HL2, Head length 2, landmarks1 to 9, HL3, Head length 3, landmarks 2 to 3, HD1, Head diagonal 1, landmark 2 to 8 and BL4, Body length 4, landmarks 6 to 7. The distance codes of *M. zebrinus* are BW2, Body width 2, landmarks 3 to 8 and BD5 and Body diagonal 5, landmarks 1-8. The distance codes of *A. testudineus* BL2, Body length 2, landmarks 4 to 5, BW3, Body width 3, landmarks 5 to 7, BD6, Body diagonal 6, landmarks 5 to 8 and BL5, Body length 5, landmarks 7 to 8. The distance codes of *T.pectoralis* HL3, Head length 3, landmarks 2 to3 and CFL1, Caudal fin length 1, landmarks 6 to 7(Table 4).

As the extraction in PC analysis, PC1 showed that the low standardized score was HL3 (-0.21) and PC2 also showed that the low standardized score was BL 4 (-0.20) component plot in rotated space also revealed the low neuroticism (HL2 and HD1) of *N. notopterus*. PC1 showed that the low standardized score was BW2 and BD5 (0.02) and PC2 also showed that the low standardized score was BW2 (0.08) component plot in rotated space, also revealed the low neuroticism (BW2) of *M.zebrinus*. PC1 showed that the low standardized score was BL2 (-0.19) and PC2 also showed that the low standardized score was BW 3 (-0.34) component plot in rotated space also revealed the low neuroticism (BD6 and BL5) of *A. testudineus*. PC1 showed that the low standardized score was HL3 (-0.05) and PC2 also showed that the low standardized score was CFL1 (-0.26) component plot in rotated space also revealed the low neuroticism (HL3 and CFL1) of *T. pectoralis* (Table 4 & Fig.2).

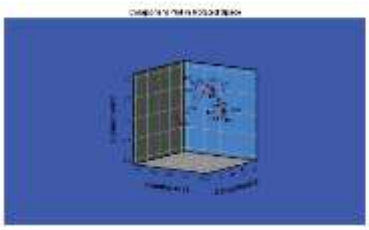
Table 4 Result of factors extraction in PC analysis after varimax normalized rotation in the study fish species

Species	Distance code	PC1	PC2	PC3
Notopterus notopterus	HL2 *	0.16	0.14	0.73
	HL3	-0.21	0.87	-0.09
	HD1*	-0.15	0.74	0.35
	BL4	0.77	-0.20	0.28
Macrognathus zebrinus	BW2*	0.02	0.08	0.76
	BD5	0.02	0.87	0.16
Anabas testudineus	BL2	-0.19	0.54	-0.01
	BW3	0.62	-0.34	0.39
	BD6*	0.09	-0.08	0.89
	BL5*	-0.01	0.26	0.83
Trichogaster pectoralis	HL3*	-0.05	0.65	0.56
	CFL1*	-0.00	-0.26	0.82

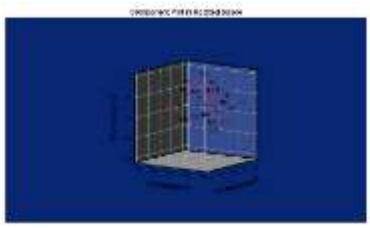
The discriminant function analysis of *N. notopterus* showed two morphological indices defining 100.0% (DF1),0.0%(DF2),0.0%(DF3) and 0.0%(DF4) as the morphological differences. The truss distances with important loading on DF1 were shown with total variance of 100.0%. The discriminant function analysis of *M. zebrinus* showed two morphological indices defining 97.4% (DF1),2.3%(DF2) and 0.3%(DF3) as the morphological differences. The truss distances with important loading on DF1 were shown with total variance of 97.4%. The discriminant function analysis of *A. testudineus* showed two morphological indices defining 91.8% (DF1),5.2% (DF2),2.2% (DF3) and 0.8% (DF4) as the morphological differences. The truss distances with important loading on DF1 were shown with total variance of 91.8%. The discriminant function analysis of *T. pectoralis* showed two morphological indices defining 93.8% (DF1),4.2% (DF2),1.2% (DF3), 0.4% (DF4), 0.3% (DF5) and 0.1% (DF6) as the morphological differences. The truss distances with important loading on DF1 were shown with total variance of 93.8% (Table 4).



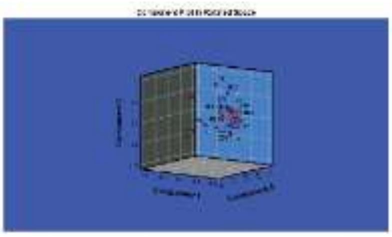
A. *Notopterus notopterus*



B. *Macronagthus zebrinus*



C. *Anabas testudineus*



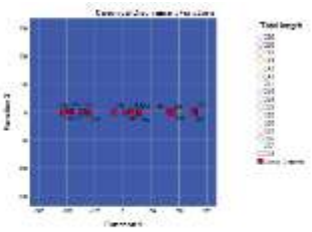
D. *Trichogaster pectoralis*

Figure. 2 Showing of score plots of the four fish species

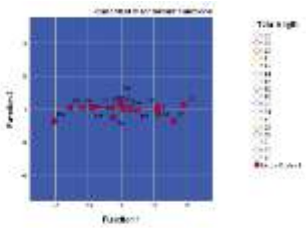
Table 5 Pooled within-groups corrections between discriminating variables and standardized canonical discriminant function of landmark distances of the four fish species

Species	DF1	DF2	DF3	DF4	DF5	DF6
Notopterus notopterus	100.0%	0.0%	0.0%	0.0%	-	-
Macrognathus zebrinus	97.4%	2.3%	0.3%	-	-	-
Anabas testudineus	91.8%	5.2%	2.%	0.80%	-	-
Trichogaster pectoralis	93.8%	4.2%	1.2%	0.4%	0.3%	0.1%

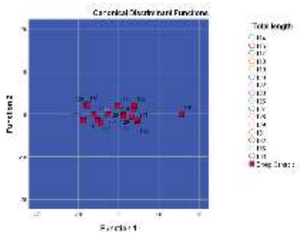
Plotting of canonical discriminant functions of *N. notopterus* showed more or less overlapping among the 20 individuals of for both morphometric (Total length) and 18 truss measurements in discriminant space. All total lengths were intermingling among population. Plotting of canonical discriminant functions of *M. zebrinus* showed a clear overlapping among the 20 individuals for both morphometric (total length) and 16 truss measurements in discriminant space. All total lengths were intermingling among populations. Plotting of canonical discriminant functions of *A. testudineus* showed more or less overlapping among the 20 individuals for both morphometric (total length) and 21 truss measurements in discriminant space. More sample total length was intermingling among each total length and some of these were less missing. Plotting of canonical discriminant functions of *T. pectoralis* showed more or less overlapping among the 20 individuals for both morphometric (total length) and 21 truss measurements in discriminant space. All total lengths were intermingling among population (Fig.3).



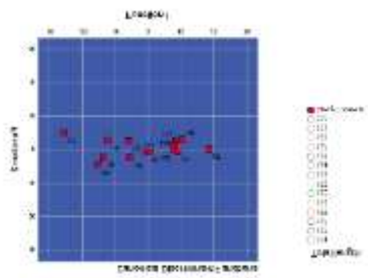
A. *Notopterus notopterus*



B. *Macronagthus zebrinus*



C. *Anabas testudineus*



D. *Trichogaster pectoralis*

Figure.3 Centroid of discriminant function score plot of the four fish species

A correct classification of individuals into their original population of *N. notopterus* varied 100% by discriminant analysis (DF1 & DF2) and 100% of individuals group cases could be classified in their correct prior grouping. A correct classification of individuals into their original population of *M. zebrinus* varied 99.7% by discriminant analysis (DF1 & DF2) and 100% of individuals group cases could be classified in their correct priori grouping. A correct classification of individuals into their original population of *A. testudineus* varied 97.0% by discriminant analysis (DF1 & DF2) and 95% of individuals group cases could be classified in their correct prior grouping. A correct classification of individuals into their original population of *T. pectoralis* varied 98.0% by discriminant analysis (DF1 & DF2) and 100% of individuals group cases could be classified in their correct prior grouping.

Discussion

Islam and Mollah (2012) expressed the efficiency of the allometric formula in removing the size effect from the data was justified by using correlation between total length and adjusted characters. That is why total length was excluded first and not transformed because using this parameter as standard all other parameters were standardized. Sathianandan (1999) also explained morphometric studies by statistical methods were based on a set of traditional measurements which were providing uneven and biased aerial coverage of the entire body form of the specimen. Truss network provides a more systematic and geometric characterization of fish shape. According to above knowledge, the present study of fish measurements was done by truss network system. Analysis of truss network measurements in the present study was done by principal component analysis (PCA). Joseph and Godwin (2000) explained that PCA does not require any prior information about the groups in the analysis of truss data. In the present study univariate analyses showed that morphometric characteristics of the study four species were significantly different among the certain group ($P < 0.05$). Wilk's lambda values of all network morphometric measurements were $0 < \lambda < 1$ so they were shown significantly differences. All 18 landmark measurements of *N. notopterus* ,16 landmark measurements of *M. zebrinus*, 21 landmark measurements of *A. testudineus* and *T. pectoralis* were found highly correlated with total length. Strauss and Bookstein (1982) reported that the factor analysis is related to principal component analysis but the two are not identical. Eigen-values of factor analysis are contaminated with error variances. Multivariate techniques simultaneously consider the variations in several characters and there by assess the similarities among the samples. The key characters used for the discrimination of the body form were those that have high eigenvalues. Thit Thit Lwin (2023) showed that the eigen-values of factor analysis are study in morphometric variation of some fish species from Weadaunt Village group of Sittaung River Basin, Bago Region based on truss network analysis. In this study, variation of eigen-values of factor analysis (FA) was found the study species. Eigen-values of two factor analysis *Mystus pulcher*, *Oreochromis mossambicus* and *Channa panaw* and three factor analysis *Mystus microthalmus.* In the present study, variation of Eigen-value of three factor analyses were *N. notopterus*, *M. zebrinus, A. testudineus* and *T. pectoralis.* The Eigen- value and factor analysis were missed the prior and present study because of the different study sites and commanded facts (value and factor). Stearns, 1983 stated that the phenotypic plasticity of fish is very high. They adapt quickly by modifying their physiology and behavior to environmental changes. These modifications ultimately change their morphology. Analysis of truss network measurements in the present study was done by principal component analysis (PCA) to know the variation of fish size and shape withing the group of fish species. In the present study, principal component analysis (PCA) was used to assess or observe the variation of within species by plotting the Principal Component scores (PC). The PCs which revealed significant differences were used for XY plots (PC1 and PC2). In the present study, the score on the scatter plot of *N. notopterus, M. zebrinus, A. testudineus* and *T. pectoralis* were indicated the size variation of network measurements in morphological and no shape effect variation in this population. Karakousis *et al.* (1991) stated that discriminant function analysis (DFA) could be a useful method to distinguish different stocks of the same species. Hossain *et al*. (2015) stated that in the discriminant functional analysis (DFA), the first DF accounted for much more of the within group variability than did the remaining other DFs. It was obvious that the other DFs explained much less of the variance than the first DF. Therefore, the other DFs were much less informative in explaining difference within the population.The present study, although the results of discriminant function analysis (DFA) of the study species were correctly classified individuals for three species 100% (*N. notopterus, M. zebrinus and T. pectoralis)* except *A. testudineus* 95% into their original population in discriminant space and from the principal component analyst's perspective, the morphological structure of only species was size variation and other three species had no size and shape variation with less missing variables in their respective populations. In the present study, *A. testudineus* was the size differentiation of the original group. They were collected and examined from the same site, Thaung Pyin "In". Therefore, observed morphological variations of study species might be due to environmental factors and genetic differences within these populations.

Conclusion

An allometric formula was used for size - adjustment transformation for all characters and multivariate analysis. including Factor Analysis, Principal Component Analysis and Discriminant Functional Analysis were utilized. Truss network analysis, in combination with these multivariate statistical methods, effectively tested and displayed morphological differences in both size and shape. The current findings provide valuable preliminary baseline information on the morphometric variation within each species in Thaung Pyin "In". Future studies should investigate whether the observed morphological differences in fish are due to genetic factors or environmental conditions. Studying the same species in different stocks in the future will offer more comprehensive insights for comparing morphometric variation across fish populations.

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