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Differentiation in morphometric traits of *Chanos chanos* stocks along the Indian coast

M. SriHari[1], Rinchen Nopu Bhutia[1], A. Kathirvelpandian[2], Rama Sharma[1], Karankumar K. Ramteke[1], G.B.Sreekanth[3], & Zeba Jaffer Abidi[1*]

[1]Department of Fisheries Resource Management,ICAR-Central Institute of Fisheries Education, Mumbai, India

[2]Peninsular Marine Fish Genetic Resources Centre, ICAR-National Buraeu of Fish Genetic Resources, Kochi, India

[3]ICAR-Central Coastal Agricultural Research Institute, Old Goa, Goa, India

*[E-mail: zjabidi@cife.edu.in]

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Stocks of *Chanos chanos* (Milkfish) are identified based on the morphometric traits from four different locations from the Indian coast. A total of 246 fish samples were collected for the present study from the East coast (Mandapam Lagoon and Chilika Lake) and West coast (Mandovi–Zuari estuarine system and Cochin backwaters). Digital imaging techniques were employed to extract 18 morphometric characters from the specimens. Principal component analysis showed that horizontal depth measurements, dorsal fin length and pelvic fin length were helpful in differentiating the milkfish populations. First and second principal components explained the variance of 37.62% and 31.26 % respectively. Cross-validated results of the discriminant analysis showed the overall classification rate of 90.4%. The present study decoded the differentiation and occurrence of separate stocks of milkfish in Indian waters. The findings of the present study could be utilized for conservation and management of the species.

[**Keywords:** Milkfish, Morphometry, Stock structure, Discriminant analysis]

Introduction

Stock is a part of a fish population usually with a particular migration pattern, specific spawning and subject to a distinct fishery[1]. Stock identification is a central theme in fisheries science that involves recognition of self-sustaining components within natural popualtions[2]. It also forms the basis for restocking and stock rebuilding programmes[3]. Stock structure of fishes can be deciphered by various methods from morphological characters, advanced morphometrics, biochemical, hard parts structure, microchemistry, parasite interactions and genetic markers. Morphological characters form a mainstay for identifying the fish stocks[4]. Negligence of stock structure studies in fisheries management can lead to the detrimental effect on the fish population in biological attributes and the genetic diversity of the species.

Morphometric technique for stock delineation includes the analysis of particular morphological features of various body dimensions or parts[5] and is the cost effective and most commonly used method for stock identification studies[6]. The study of morphological characters with the aim of differentiating the fish stocks has a keen interest in ichthyology[7]. Environmental factors tend to affect the morphometric characteristics of the stocks. Phenotypic variations are mainly attributed to their differential geographic distribution and isolation. Environmentally induced phenotypic variation has its own advantage in differentiating the stocks; especially when insufficient time for significant genetic difference accumulates between the stocks[8]. Stock delineation using morphometric markers gives us the perspective of evolution, ecology, behavior, conservation, water resource management, and stock assessment[9].

Chanos chanos (Milkfish) is a monotypic species of the family Chanidae widely distributed in the tropical Indo-Pacific region. Milkfish is a euryhaline migratory species, which occurs in littoral waters[10] and found along the latitudinal range of coral reefs that are restricted to shallow, clear and warm water > 20˚C[11]. Adult milkfish are powerful swimmers, which usually spawns in the open sea and release planktonic eggs[12]. Milkfish larvae migrate from the open sea to shallow coastal waters where the abundance of food is available for growth. The habitat areas, depth and their connectivity with the sea influence duration of stay of milk fish in the coastal waters[12]. Milkfish is one the best candidate species for aquaculture in developing countries like India. In 2015, captive breeding and seed production protocol for this species were standardized in India[13]. Since breeding protocol has been standardized, there is a need to study the stock structure of the species in Indian waters that help in the improvement of future breeding programmes.

Population studies on milkfish have been conducted in South-East Asian countries by using morphological markers[14,15,16]. The study of vertebral number in milkfishes of South and South-East Asia has shown four existing populations *viz.,* Indian, Thai, Philippines-Taiwan-Indonesian, and Tahiti[14]. Since only one sampling location was taken along Indian coast in the previous study, it is hard to conclude information about milkfish stocks in Indian waters. No extensive studies are available identifying the stocks of milkfish in Indian waters. Hence differentiation of *C. chanos* stock has been attempted which will be helpful in selective breeding and stock rebuilding programmes in the near future.

2. Material and Methods

2.1 Sampling

Specimens of *C. chanos* from the Indian waters were collected from Mandapam lagoon (Tamil Nadu), Chilika lake (Odisha) in East Coast and Cochin Backwaters (Kerala), Mandovi-Zuari estuarine system (Goa) in West coast (Fig. 1). A total of 246 samples were collected between September 2016 to January 2017.

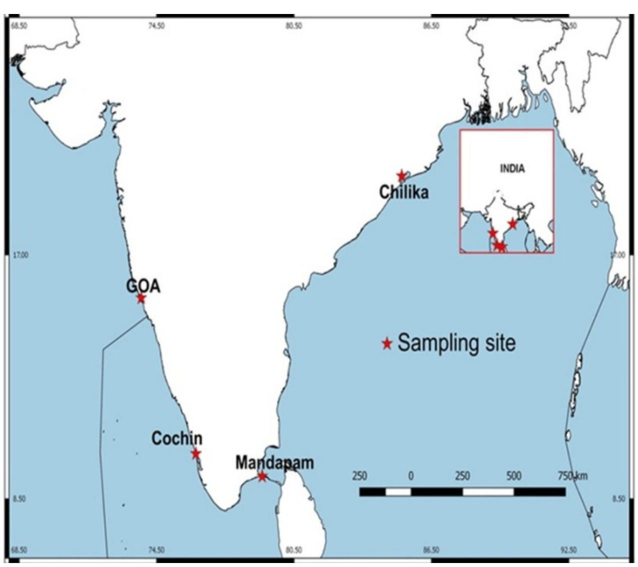

Fig. 1 — Sampling sites

The specimens without physical damages were collected randomly and packed in insulated styrofoam boxes for transportation to the laboratory.

2.2 Extraction of morphometric measurements

Image of fish specimens was captured by using Canon Powershot-SX410 IS. A total of eighteen morphometric measurements were used in this present study (Table 1 and Fig. 2). They were extracted by using three softwares, the images were converted from .jpg format to tps format by using tpsUtil[17]. The converted tps image file is further loaded into the tpsDig2 V2.1[18] software to digitize the predetermined landmarks. The landmark data were encrypted into tps files as X-Y coordinates and measurements between the landmarks were extracted by using Paleontological Software (PAST) [19].

Table 1 — Morphometric characters of *Chanos chanos* used for present study

S.No.	Morphometric traits
1	Total length
2	Standard length
3	Fork length
4	Head length
5	Pre-Orbital length
6	Eye Diameter
7	Post-Orbital length
8	Pre-Dorsal length
9	Dorsal fin base length
10	Dorsal fin length
11	Pre pectoral length
12	Pectoral fin length
13	Pelvic fin length
14	Pre-Pelvic length
15	Pre anal fin length
16	Anal fin base length
17	Maximum body depth
18	Caudal peduncle depth

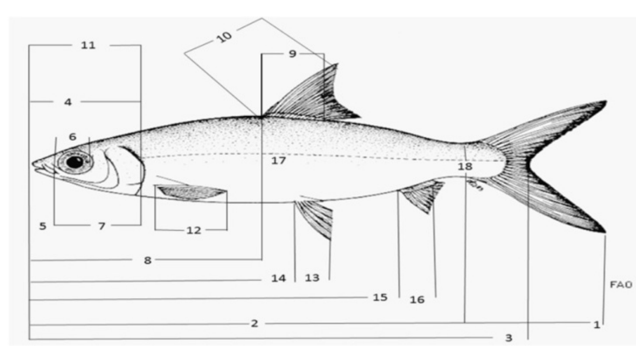

Fig. 2 — Image illustrating the morphometric traits used in this study

2.3 Removal of size-dependent effects

The data were checked for outliers and 37 outliers were removed and 209 samples were finally selected. A significant correlation was found between the body size and morphometric variables. 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[20]

$$\mathbf{Mtrans = M\ (Ls/Lo)^{b}}$$

Where,

Mtrans = transformed truss measurement/ transformed morphometric measurement

M = original truss measurements/ original morphometric measurement

Lo = standard length of fish

Ls = overall mean of standard length

b = within group slope of the geometric mean regression calculated with log-transformed variables, M and Lo

Descriptive statistics *viz.,* minimum, maximum, mean, standard error of mean and co-efficient of variation were estimated for extracted morphometric traits. Data were checked for normal distribution. Multivariate Analysis of Variance (MANOVA), Principal Component Analysis (PFA) and Discriminant Function Analysis (DFA) were performed to assess the significant variation among the morphometric traits between the stocks. STATISTICA[21] software package was used to perform the above mentioned statistical analysis.

Results

Descriptive statistics of 18 morphometric traits were given in Table 2. The total length of this species ranged from 15.40 to 34.17 cm with a coefficient of variance 22.11%. The maximum total length was observed in a Goa specimen. After removing the size-dependent variations, none of the measurements show significant correlation with the standard length of fish, which clearly show that effect of body length has been removed.

Multivariate analysis of variance was carried out for morphometric traits and it shows significant difference between the stocks from four locations (Wilk's lambda = 0.0, $F = 33.9$, $p < 0.05$). The principal component analysis revealed significant morphometric variation of stocks in Indian waters. The principal components with an eigenvalue above 1 were included and others were excluded in this study. The first three principal components together explained 75.70% variation. Among which first, second and third components explained 37.62%, 31.27% and 6.8% respectively. Factor loading more than 0.30 is considered significant, 0.40 is considered more significant, and 0.50 and above is considered very significant[22]. In our present study, only those

Table 2 — Descriptive statistics of morphometric traits

Traits (cm)	Minimum	Maximum	Mean±SE	CV%
Standard length	12.37	25.21	19.06±0.26	20.38
Fork length	13.64	28.13	21.25±0.30	20.63
Total length	15.40	34.17	24.99±0.38	22.11
Pre-Orbital length	0.49	1.40	0.95±0.01	24.06
Eye diameter	0.72	1.76	1.19±0.01	19.77
Post-Orbital lngth	1.51	3.66	2.40±0.03	20.88
Head length	2.90	6.41	4.53±0.06	19.95
Pre-Pectoral length	3.34	7.47	5.20±0.07	20.81
Pectoral fin length	1.07	4.56	2.73±0.06	32.96
Pre-Dorsal length	6.67	14.16	10.28±0.13	19.36
Dorsal fin base length	1.41	3.57	2.54±0.04	23.70
Dorsal fin length	1.35	4.52	3.06±0.06	30.47
Pre pelvic length	7.55	15.81	11.75±0.15	19.13
Pelvic fin length	1.15	3.68	2.35±0.04	29.89
Pre-Anal length	10.32	21.31	16.09±0.22	19.99
Anal Fin Base length	0.72	1.97	1.34±0.02	24.66
Caudal peduncle length	1.03	2.26	1.68±0.02	21.60
Maximum body depth	2.52	6.48	4.71±0.08	24.89

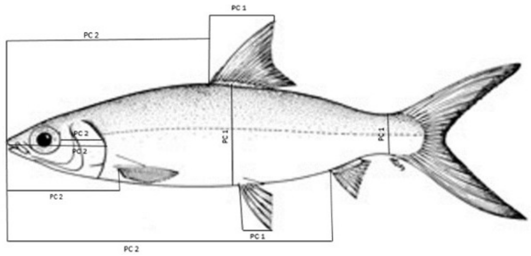

Fig. 3 — Morphometric variables showing high loadings on PC1 and PC2

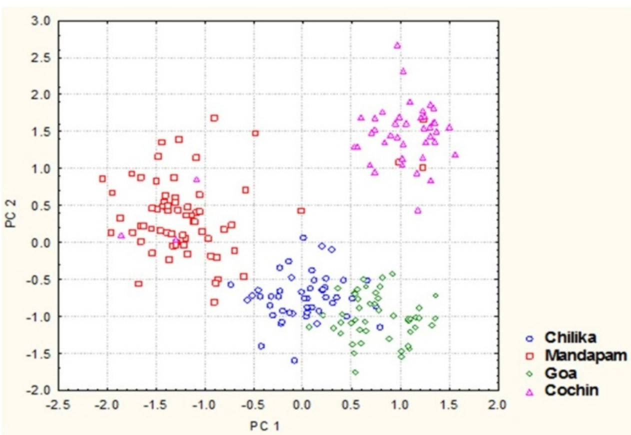

Fig. 4 — Scatterplot of PC1 and PC2 from principal component analysis

factors with loadings greater than 0.70 were considered. The variable with high loadings on the first two principal components was helpful in differentiating the stocks.

The first principal component (PC 1) was highly loaded on the dorsal fin length, pelvic fin length, caudal peduncle depth and maximum body depth while the second principal component (PC 2) was loaded on post orbital length, head length, pre-pectoral length, pre-dorsal length and pre-anal length (Fig. 3). The location wise scatter plot of PC 1 and PC 2 depicted a separation of all four populations along the horizontal axis which depicts the contribution of PC 1 in separation There was a high degree of separation between the Cochin and Mandapam populations and there was a slight overlap of Goa and Chilka populations (Fig. 4)

The variables, which are highly loaded on the first two components, have been taken for discriminant function analysis. Location-wise discriminant function analysis has given overall classification rate of 90.4%. (Table 3)

Table 3 — Cross-validated classification from discriminant function analysis

Number of observations and percent classified into locations

Location	Chilika	Mandapam	Goa	Cochin	Total
Chilika	44	0	5	0	49
	89.8	0	**10.2**	0	**100**
Mandapam	0	63	0	4	67
	0	**94**	0	**6**	**100**
Goa	8	0	42	0	50
	16	0	**84**	0	**100**
Cochin	0	3	0	40	43
	0	**6.98**	0	**93.02**	**100**

Total Success classification rate: 90.4%

Discussion

The results obtained from the present study deciphered the differences in milkfish stocks along the Indian coast. In general, fishes show a higher degree of variation within and between populations than other vertebrates and are more susceptible to environmentally induced variation[23].

The fishes were found to be differentiated mainly on the depth measurements. Studies along Indian waters have well reported that depth measurement as the major factor which helps in the differentiation of *Decapterus russelli*[24], *Megalaspis cordyla*[25] and *Nemipterus japonicus*[26] stocks. Caudal peduncle depth and Body depth were helpful in contributing to the separation of milkfish populations in the pacific ocean[15]. It is found that gut diameter and body depth were positively correlated with respect to feeding intensity[27]. Diet-induced morphological divergence was recorded earlier in bluefish[28]. The increase in the size of fish along the West coast may be the result of the intrusion of upwelled waters in Cochin Backwaters and Mandovi-Zuari estuarine system. Arabian sea is considered to be one of the top most productive regions of the world due to upwelling, wind-driven mixing and lateral advection[29] The intrusion of nutrient-rich upwelling waters in these areas were well documented [30,31]. Morphological variation related with the body depth can be related to the abundance of food, which in turn results in increasing feeding intensity.

Use of dorsal fin in swimming patterns of coral reef fishes was well reported[32] and also increase in the size of the fins of *Salmo salar* with respect to the current velocity were studied[33]. Since milkfish spawns on coral reef areas, the differentiation of dorsal fin length may be due to the velocity and current patterns available in respective regions. Milkfish juveniles used to take the feed from the bottom[12] and their pelvic fin needs to be longer to produce sufficient upward force to move the fish to near bottom. The pelvic fin length variations can be related to the deeper Cochin backwaters and Mandovi-Zuari estuarine system when compared to its counterparts in East coast. Hence, the longer pelvic fin of the fishes in West coast may be due to the increase in depth of respective locations.

The variation in caudal peduncle region of the fishes from the East and West coast could be a consequence of phenotypic plasticity in response to the uncommon hydrological condition between locations. Differences in the size of the caudal peduncle with respect to the prevailing current velocities is well studied. The morphological difference in caudal peduncle region of brook char from different water velocities is observed and deeper caudal peduncle was observed in fishes from turbulent waters[34]. Increase in caudal peduncle depth is related to the turbulent waters of the west coast due to its direct connectivity with sea without a sand barrier, which is present along the Chilika and Mandapam sites. Variation in the head related morphometric characters may be attributed to the differential habitat use and based on the prey size[35].

Lesser differentiation between Chilika and Goa stocks may be due to the prevalence of similar environmental parameters between these two regions, which had shown the elongated body due to its increase in per-anal, pre-dorsal and pre-pectoral length. Lack of shape differentiation between the Goa, Chilika and Kakinada stocks of *Lates calcarifer* has been observed based on truss network analysis[36].

Conclusion

It may not be clear from the level of study that site-specific environmental parameters have been influencing the morphological differentiation of four stocks along the Indian coast; and collection of environmental data would have made it clearer. Population genetic structure of the species have to be studied in future to increase the validity of the present study.

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