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RESEARCH PAPER

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Deciphering the Stock Structure of *Chanos chanos* (Forsskål, 1775) in Indian Waters by Truss Network and Otolith Shape Analysis

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Abstract

Chanos chanos is one of the Indo-West Pacific fish species normally found along the Indian coast. Because the breeding protocol for milkfish has been standardized in India, there is an urgent need to study the stock structure of the species to select the best traits for future breeding programs and to conserve the species. A total of 246 fish samples were collected from four locations, Chilika Lake and Mandapam lagoon, in the East coast and Cochin Backwaters and Mandovi - Zuari Estuary, in the West coast of India to delineate the stocks of Milkfish along the Indian coast. A total of 21 truss distances and five otolith shape indices were measured. Principal component analysis was conducted for truss and otolith data. Mid-body depth and caudal peduncle depth measurements were highly useful in discriminating the stocks. All shape indices differed significantly between the sampling locations. Cross-validation by discriminant analysis of morphometric traits revealed that 87.6% of the individuals were correctly classified into their respective locations, while otolith shape data classified 59.6% of the fish samples correctly to their sampling sites**.** This study revealed that there is the existence of different populations of this species at the respective sampling locations. Future studies should focus on delineating the populations from all the geographical locations along the Indian coast.

Introduction

Despite the increase in the fishing effort over a period, the world capture fisheries production remains static for the past few decades but resulted in overfishing, habitat degradation and economic unsustainability. To increase the fish productivity and to ensure a supply of protein-rich food to the human population, ranching of hatchery-reared juveniles of fish is considered as the best alternative. A proper knowledge of fish stocks will help us in the management, conservation of endangered species and stock enhancement of cultivatable species.

Stock is a part of a fish population usually with a particular migration pattern, specific spawning grounds and subject to a distinct fishery (ICES, 2012). Fish stocks can be identified by various methods *viz*. morphological characters, advanced morphometrics, biochemical signals, the structure of hard parts, microchemistry, parasite interactions and genetic markers. In the context of interdisciplinary stock identification, the morphometric analysis provides information on phenotypic stocks, which has similar growth, mortality, and reproductive rates (Booke, 1981). As the traditional morphometric measurements have biased coverage over the body (Strauss & Bookstein, 1982), the truss network system is the best alternative, which covers the fish in a uniform network and increases the likelihood of extracting morphometric differences within and between species (Turan, 1999). In as much as, otoliths remain unaffected by short-term changes in fish condition, it can be considered to be a tool for species discrimination, stock analysis, and even in testing the function and ecological significance of shape differences in the studies of otolith morphology (Cardinale, Doering-Arjes, Kastowsky & Mosegaard, 2004).

Chanos chanos (Forsskål, 1775) is a monotypic species of the family Chanidae, occurring in marine and brackish waters. Milkfish is distributed throughout the South and Southeast Asia and is one among the few Indo-west Pacific species occurring along the East Pacific Barrier (Bagarinao, 1991). In India, a conservative estimate states that at least 20 million wild-caught seeds of the species are collected every year for farming purposes (MPEDA, 1997) and it could cause deleterious effects on the recruitment of the natural population of milkfish. Milkfish populations along south-east Asian countries were studied using various morphological and genetic markers, such as traditional morphometric data (Winans, 1985), meristics (Senta & Kumagai, 1977; Villauz & MacCrimmon, 1988), electrophoretic variation (Winans, 1980), AFLP (Adiputra, Chuang & Gwo, 2012) and RFLP (Ravago-Gotanco & Juinio-Menez, 2004) and distinct stocks of milkfish have been inferred from the Southeast Asian countries, such as the Philippines and Indonesia. To formulate the management strategies, policy regulations and for conservation of the species, a proper understanding of the stock structure of milkfish species in its areas of distribution is a prerequisite. The present study will also be a valuable baseline investigation for describing changes in shape features to identify the differences between the wild and farmed stocks of milkfish in the future. However, so far, no stock identification studies have been carried out in the milkfish populations for Indian waters, and this present work will be the first to characterize the stocks of milkfish in Indian waters by using truss network and otolith shape indices.

Materials and Methods

Sampling

Specimens of *C. chanos* were collected from four areas, *viz.* Mandapam lagoon (9˚16' N, 79˚7' E) in Tamil Nadu and Chilika Lake (19˚ 74' N, 85˚ 21' E) in Odisha represents the East coast, and Cochin Backwaters (9˚59' N, 76˚14' E) in Kerala and Mandovi - Zuari estuarine system (15˚ 50' N, 73˚ 83' E) in Goa represents the West coast (Figure 1). A total number of 246 samples were collected between 2016 and 2017 (Table 1). The specimens without physical damages were collected randomly and packed in insulated styrofoam boxes for transportation to the laboratory.

Digitization of Samples

The specimens were washed thoroughly with fresh water, wiped, placed on a laminated graph sheet, and

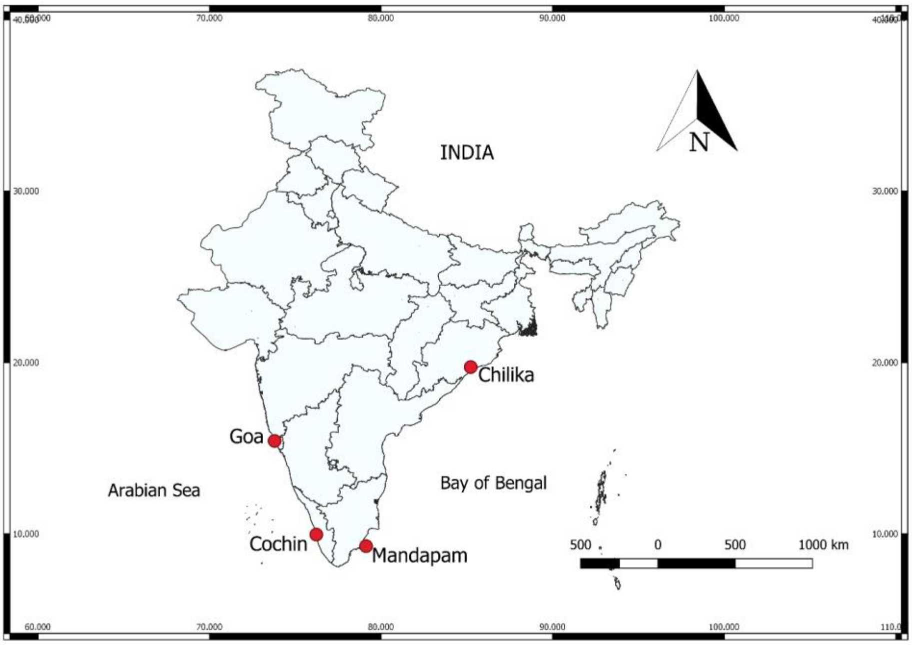


Figure 1. Sampling locations of *Chanos chanos* from the Indian coast.

fins were erected in their natural position; then photographed using a high-quality camera (Canon Powershot-410 IS) mounted on a tripod stand to give steady images.

In the laboratory, left sagittal otoliths were collected using a scalpel, washed in running water to remove the flesh, dried overnight and stored in vials. Otolith images were captured using a stereo zoom microscope Olympus SZX16 (Figure 2). The microscope magnification was adjusted to the size of the otolith to ensure the highest resolution possible, varying between 1.5X and 2X.

Measurement of Truss Distances and Otolith Shape Indices

A truss network was constructed on the fish surface using ten homologous anatomical landmarks. A total of 21 interconnecting measurements were obtained using the truss network (Figure 3). Measurements were extracted using the linear combination of two software tpsDig2 (Rohlf, 2006a) and Paleontological Statistics (PAST) (Hammer, Harper & Ryan, 2001). The files were converted from jpeg format to tps format by using tpsUtil (Rohlf, 2006b). The

Table 1. Details of *Chanos chanos* samples collected from various locations in the East and the West coast of India

Coast	East Coast		West Coast	
Sampling sites	Chilika	Mandapam	Cochin	Goa
No.of. samples (Truss Network Analysis)	49	67	43	50
No.of. samples (Otolith Shape Indices)	41	47	38	40

Figure 2. Image of the left sagittal otolith.

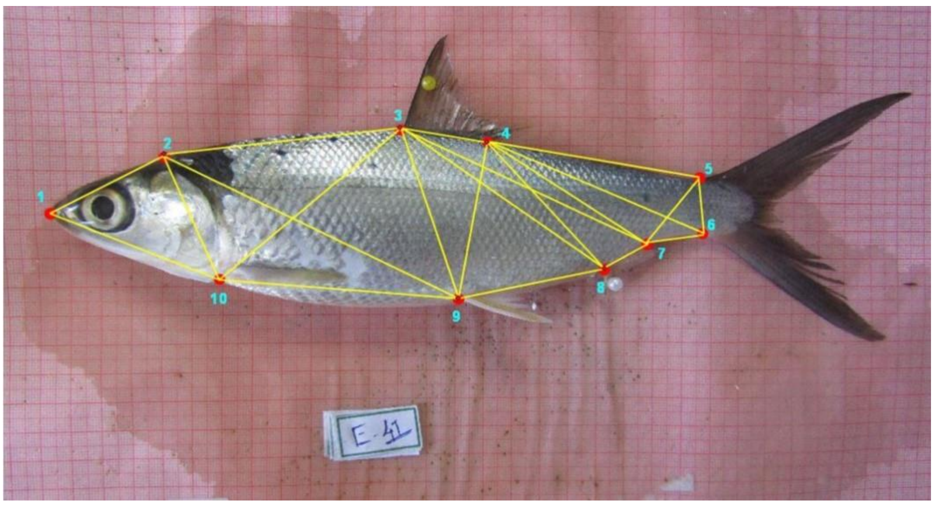


Figure 3. 10 point Truss network of *Chanos chanos.*

converted tps image file was further loaded into the tps Dig2 software to digitize the predetermined landmarks. The landmark data were encrypted into tps files as X-Y coordinates. Paleontological Statistics (PAST) software was used to extract the distance between the landmarks.

The digitized images of otoliths were analyzed using Sigma Scan Pro Version 5.0.0 image analysis software to measure its area (Ae), perimeter (Pe), maximum length (Le) and maximum width (We). Otolith shape indices, including circularity, ellipticity, rectangularity and form factor (Table 2), were then calculated using the method of Tuset, Lozano, Gonzalez, Pertusa and García-Díaz (2003).

Analysis of Data

To overcome the size-dependent variations resulted from the allometric growth of fish, the absolute measurements of fish and otolith were transformed into size independent variables using the formula (Elliott, Haskard & Koslow, 1995)

$$M_{trans} = M\,(L_s / L_o)^{b}$$

Where,

M_{trans} = transformed truss measurement / transformed morphometric measurement

M = original truss measurements / original morphometric measurement

L_o = standard length of fish

L_s = overall mean of standard length

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

Fish data were tested for normality check, and 37 outliers were removed. A total of 209 samples were finally selected for the statistical analysis. Multivariate analysis of variance (MANOVA) was performed for truss measurements and otolith shaped indices to test the significant difference between different locations of sampling. The data reduction method, principal component analysis (PCA) was applied to morphometric data in order to extract the factors, which are important in discriminating the populations of milkfish. The factors with a loading score of more than 0.30 is considered as significant, > 0.40 is more significant, and loading scores of 0.50 and above are considered very significant (Lombarte, Gordoa, Whitfield, James & Tuset, 2012). Therefore, the truss distances which had loadings > 0.70 were selected and subjected to discriminant function analysis (DFA), which classifies the fish samples to their respective locations based on their shape differences (Pazhayamadom *et al.*, 2015). The DFA combines the factors in a linear fashion to produce a mathematical function that can be used to classify individuals into separate groups (Turan, Ergüden, Gurlek & Turan, 2004). Statistical analyses were performed using STATISTICA 8.0 software package.

Results

Truss Network Analysis

Multivariate analysis of variance was carried out to determine the significant difference between the populations. Wilk's lambda, Pillai's trace, Hotelling and Roy test have demonstrated significant differences between the locations ($p < 0.05$). In PCA, the first three principal components explained 71.49% of the total variation in the data; with first, second and third principal components contributing 43.53%, 19.28%, 8.67% of the total variation, respectively. The truss distances that had loadings > 0.70 were selected. The variables 3-7, 3-8, 3-9, 4-9, 5-6 and 8-9 had the highest loading on the PC 1. These truss distances concentrated on middle body region and caudal peduncle region of the fish. The variables 1-10, 4-5, 4-6 and 4-7 loaded on PC 2 were concentrated on the head and body portions between the posterior end of dorsal fin to insertion of the anal fin, anterior and posterior origin of the caudal fin (Figure 4). Distances with meaningful loadings on the first two principal components in truss network analysis of the species were given in Figure 4. The location wise scatter plot of PC1 and PC2 separated the Cochin and Mandapam populations, while a high degree of morphological homogeneity was observed between Chilika and Goa populations (Figure 5). The characters with high loadings in principal components like 1-10, 2-9, 3-7, 3-8, 3-9, 4-9, 5-6, 4-5, 4-6, 4-7, and 8-9 were taken for discriminant analysis. The misclassification rates were 22.4%, 9%, 12%, and 6.9% for Chilika, Mandapam, Goa, and Cochin, respectively. Overall classification rate was estimated as 87.6% (Table 3).

Table 2. Size parameters and Size descriptors used for identification of *Chanos chanos*

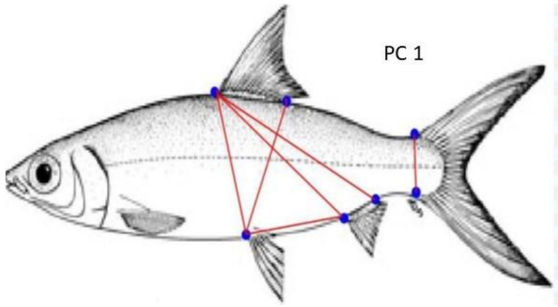
Size parameters	Shape Indices
Area (Ae)	Circularity = $(Pe^2)/Ae$
Perimeter (Pe)	Ellipticity = (Le-We)/(Le+We)
Width (We)	Rectangularity = Ae/(Le*We)
Length (Le)	Form Factor = $(4\pi Ae)/Pe^2$
	Roundness = $(4Ae)/(\pi Le^2)$

Otolith Shape Analysis

The circularity and ellipticity were found to be lowest in the Mandapam stock, whereas the form factor and roundness were highest in Mandapam stock, compared to other three stocks; but the readings of rectangularity among the stocks were more or less similar, rather complex. All the 5 shape indices used in this study have shown significant differences between the populations, which agreed with the MANOVA results. Scatterplot of PC1 and PC2 showed similarity to the scatter plot of the truss network analysis (Figure 6). Jack-knifed classification revealed a higher rate of classification of individuals into Mandapam (70.2%), while the lower rate was observed for Chilika samples (39%) (Table 3)

Discussion

Earlier studies on milkfish along the Indian coast were based on its food and feeding habits (Chacko, 1945), spawning ground and seasonal abundance of fry (Silas, Mohanraj, Gandhi & Thirunavukkarasu, 1980), biology and biometry (Gandhi, Mohanraj & Thiagarajan, 1986) and seed collection sites (Dorairaj *et al.*, 1984). No previous studies were available exclusively on the stock



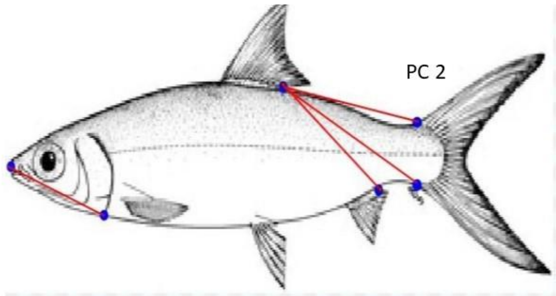


Figure 4. Distances with the meaningful loadings on the first three principal components in truss network analysis of *Chanos chanos.*

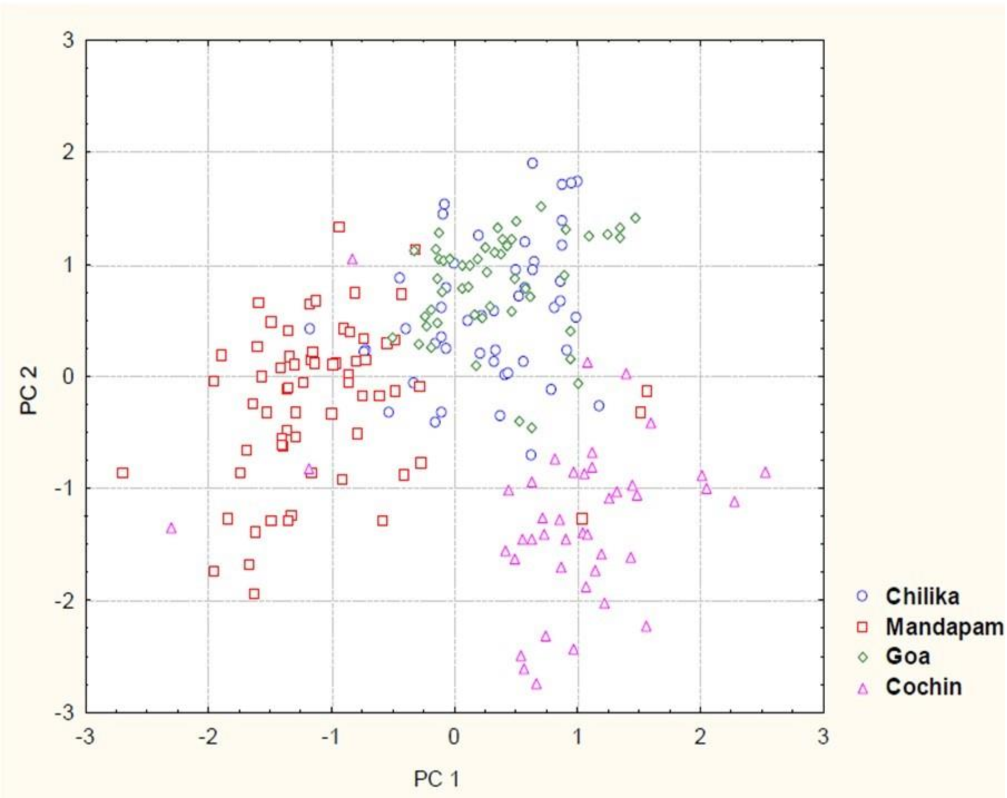


Figure 5. Scatter plot of scores of the PC1 and PC2 extracted from truss distances of *Chanos chanos* from all locations.

Table 3. Jack-Knifed percentage classification matrix for truss network analysis and otolith shape indices of *C. chanos* in the Indian waters

Location	Chilika	Mandapam	Cochin	Goa
Chilika	77.6 (39.0)	4.1 (17.1)	0 (7.3)	18.4 (36.6)
Mandapam	4.5(21.3)	91(70.2)	4.5(2.1)	0(6.4)
Cochin	0 (5.3)	6.98 (5.3)	93.02 (68.4)	0 (21.1)
Goa	12 (27.5)	0 (2.5)	0 (10)	88 (60)

*Values in parenthesis represents the percentage correctly classified for otolith shape indices in respective locations.

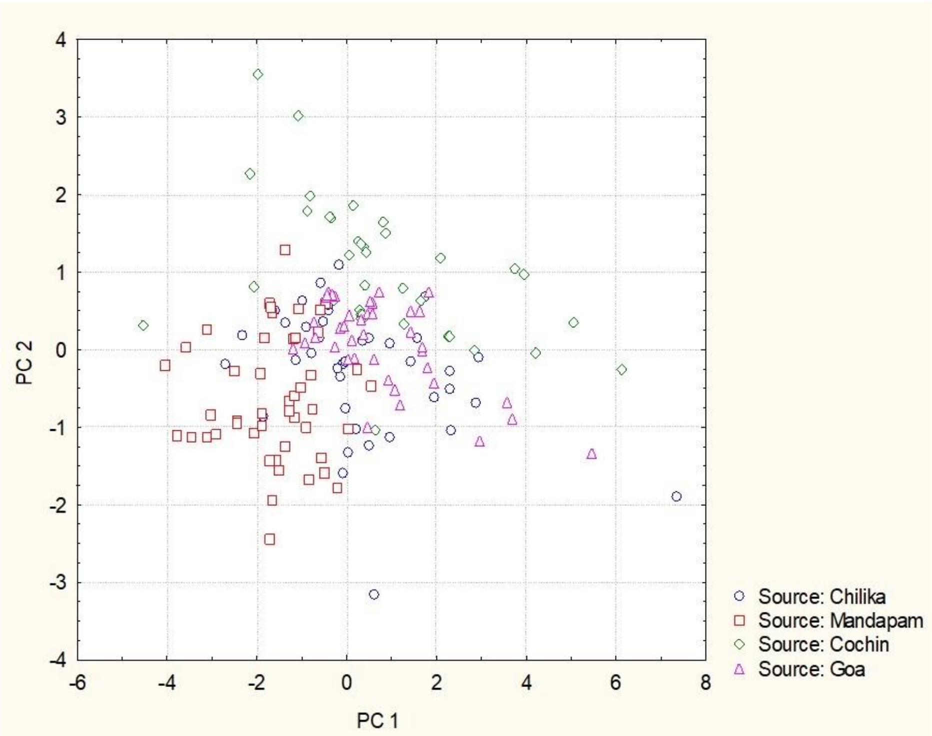


Figure 6. Scatter plot of scores of the PC1 and PC2 extracted from Otolith shape indices of Chanos chanos from all locations

structure of milkfish populations along the Indian coast except for two studies carried out by Senta and Kumagai (1977) and Ravago-Gotanco and Juinio-Menez (2004) in which samples were collected only from a single location along the Indian coast. This study is the first to analyze the stock structure of Milkfish from different locations along the Indian coast, which could help in the development of scientific management strategies for sustainable utilization of this species. In the following sections, we discuss the possible causes for the morphometric variations and changes in otolith shape between stocks of Milkfish.

Truss Network Analysis

The PC1-PC2 plot showed clear morphotype separation for Cochin and Mandapam stocks on the horizontal axis and a complete mixing of Chilika and Goa stocks. The most substantial differences were observed for the mid-body and caudal peduncle regions. The shape contributors, such as body depth and caudal peduncle depth form the major cause of variation of stocks in this study. Truss measurements were found to be similar to the results obtained from the previous study in Philippine waters (Winans, 1985). The mid-body region helped in the separation of stocks of *Decpterus russelli*, *Harpodon nehereus*, *Nemipterus japonicus* along the Indian coast (Sajina, Chakraborty, Jaiswar, Pazhayamadom & Sudheesan 2011; Pazhayamadom *et al.*, 2015; Sreekanth, Chakraborty & Jaiswar, 2017). Cavalcanti, Monteiro and Lopes (1999) had also reported similar factor loading on the first component, while analyzing the morphometry of serranid species using PCA. Upwelling on the west coast of India during the south-west monsoon, increases the food availability for fishes (Rao, Ramamirtham, Murty, Muthuswamy, Kunhikrishnan & Khambadkar 1992). The availability of Larger specimens of fish in the west coast of India would be attributed to this increased availability of food (Sreekanth *et al.*, 2017).

The significant morphometric variation between the stocks of Mandapam and Chilika might be due to the presence of coral reef structures (Sajina *et al.*, 2011). The high productivity in the coral reef areas may result in the formation of a separate stock in Mandapam. The variation in the caudal region may be the result of the turbulence in water along the two different coasts, which is also responsible for the separation of the populations of Cochin and Mandapam waters (Sajina *et al.*, 2011). Imre, McLaughlin and Noakes (2002) had also reported deeper caudal peduncle in *Salvelinus fontinalis* and the increased depth in the caudal peduncle was found to be associated with more turbulent water conditions.

The present study showed that there are no shape differences between the Chilika and Goa stocks. Gopikrishna, Sarada and Sathianandan (2006) also reported the same, between Chilika, Goa and Kakinada stocks of Asian seabass, *Lates calcarifer*. Most of the variables, including oblique depth and caudal region, which contributed to the stock identification study of sea bass, were similar to the findings of the present study. Hence, the similarity in Goa and Chilika stocks solely depends upon the environmental parameters and food abundance, which contributed to the similarities in body depth measurements. Similarly, no shape differences of populations from East and West coast were observed in *Megalapsis cordyla* (Sajina *et al.*, 2011) and *Nemipterus japonicus* (Sreekanth *et al.*, 2017); but these populations were well separated along with other locations in its respective coast. Physical characteristics of the habitats influence modify the morphological attributes of the fish populations in the geographical location (Haas, Blum & Heins, 2010)

Discriminant function analysis was a useful method for spatial distribution of fish stocks (Karakousis, Triantaphyllidis & Economidis, 1991). The stock delineation of brackish water fish, *Liza abu* revealed 100% classification success on Tigris, Euphrates and Orontes stocks (Turan *et al.*, 2004). Thus, the high classification rate of 86% may be related to the habitat of the residing population enclosed or leaves only a small connectivity with the sea and also, possibly, because of the high variation of environmental parameters between the enclosed areas, rather than in open sea.

Otolith Shape Analysis

The principal components plot showed the similarities of Goa and Chilika samples, while Cochin and Mandapam samples were well separated on the vertical axis. The differences in the surrounding water temperature and nutritional condition have been attributed to the separation of wild and cultured stocks of *Gadus morhua,* while analyzing the differences in otolith morphology (Cardinale *et al.*, 2004). Fluctuations of the diet in terms of quantity and frequency also determine the shape of the otolith over a short period of time (Gagliano and McCormick, 2004). The growth rate of fish has a direct impact on the size and shape of the otoliths (Gauldie and Nelson, 1990). The variation in food availability, effect on various environmental parameters with respect to growth rate results with changes in the shape of the otolith along both coasts. Otolith shape variability could be associated with the changes in the feeding level and growth, which also determines the body shape (Rodgveller, Hutchinson, Harris, Vulstek & Guthrie III, 2017). Relatively smaller otoliths from the East coast of India are the result of the slow growth rate of fishes in the west coast; however, the factors influencing the changes in the specific otolith shape indices were not clearly understood (Burke, Brophy & King, 2008). Otolith shape could be similar in fish inhabiting in the same ecological conditions and might varies with the difference in the habitat (Parmentier, Vandewalle & Lagardère, 2001). Otolith shape variability may be caused by several factors related to genetic (Tuset *et al.*, 2003), ontogenetic and environmental factors such as temperature, habitat, seasonal variations and diet (Campana, 2001), and ecological and biological behaviour of the species (Tuset *et al.*, 2003). Cross-validation of discriminant analysis of otolith shape data demonstrated 59.6% success rate in classification of individuals to their respective locations. The overall analysis also showed a reasonably fair classification rate for different locations. The comparative study of the body morphometry and otolith shape data on *Sardinops sagax* described lower classification rates of otolith data from body shape analysis (Vergara- Solana *et al.*, 2013). In the present study also, the classification success of otolith shape analysis was lower less compared to the rate of classification based on morphometric data. The sources of misclassification in the analysis of otoliths may include methodological inaccuracies, individual variability and migration (Campana and Casselman, 1993; Tracey, Lyle & Duhamel, 2006)

Morphotype differences, along with spatial differences, could be caused by different environmental conditions, such as temperature, salinity, depth, water masses, current and food availability for fish (Tsujita & Kondo, 1957; Sen, Jahageerdar, Jaiswar, Chakraborty, Sajina & Dash, 2011) or differences in genetic makeup (Khan, Miyan & Khan, 2013). Geographical separation and isolation of year classes at early life stages causes the morphometric variability of different stocks (Swain, Hutchings & Foote, 2005). Identification of stocks by morphological markers might be appropriate for the management of fisheries resources, even if the phenotypic diversity is not reflected in genetic diversity (Cadrin, 2000) and genetic markers may not be useful enough to reveal the morphological differentiation in stocks because only a small quantity of DNA was being analyzed (Turan *et al.*, 2004). The effective strategy for stock identification was to integrate results from different methods to form conclusions on the population structure. The present study used two different methods that are consistent with each other in discriminating the stocks of milkfish in Indian waters, and the results of the methods employed were similar, indicating the presence of separate stocks of milkfish in Indian waters.

Nine different milkfish populations have been identified by using both protein electrophoretic and morphological data (Bagarinao, 1991). Vertebral number differentiated the Indian population of milkfish from Thai, Philippine-Taiwan-Indonesian and Tahitian populations (Senta & Kumagai, 1977). This study provides us the base line information on the stock structure of milkfish that revealed the existence of three groups in India waters and among which two of the sampled populations possess similar body morphology. A detailed study on the growth and reproductive traits of milkfish could provide a clear picture and insight into the selective breeding programs of traits with superior performance. From this study, it was concluded that shape studies for stock discrimination could be treated as a reliable method. Further, stock delineation using genetic markers must be used to vindicate the findings of the study. Results of the present study will help to formulate stock specific management practices and selective breeding programs for the species in the near future.

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