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Stock structure analysis of oil sardine *Sardinella longiceps* (Valenciennes, 1847) from southeast and southwest coasts of India

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Original Article

Abstract
A total of 200 specimens of oil sardine *Sardinella longiceps* collected from Kochi in the southwest coast and Chennai in the southeast coast were subjected to truss analysis. A truss network was constructed by interconnecting 10 landmarks to form a total of 21 truss distance variables extracted from the landmarks. The transformed truss measurements were subjected to factor analysis which revealed that there is no separation of the stocks along southeast and southwest coasts. The marginal differences in shape and form are attributed to the ecological differences in the habitats which are evident from differences in length weight relationships and feeding intensity of the population along these two coasts.

Keywords: *truss network analysis, stock structure, phenotypic homogeneity*

Introduction

The oil sardine, *Sardinella longiceps* forms one of the major single species fisheries contributing about 0.72 million tons along the Indian coasts (CMFRI, 2013). Knowledge on the stock structure of the target species is fundamental to formulate resource management plans and for sustaining the marine fish stocks (Shaklee and Bentzen, 1998). During the last 10 years, oil sardine abundance has increased along the southeast coast which is stated to be an impact of changing climatic conditions (Vivekanandan, 2011). It is not clear whether the oil sardine populations of the southwest and southeast coasts are from a single stock. Therefore there is a need to assess the stock structure of the species along the southeast and southwest coasts of India.

Truss network analysis (Strauss and Bookstein, 1982) is a quantitative method for describing the shape of fish (Cavalcanti *et al.*, 1999; Sen *et al.*, 2011) by measuring the morphometric variations between species and also between stocks of a species (Turan, 1999). This system is more effective in identifying stocks and differentiating species in comparison with the traditional morphometric methods. For example Sajina *et al.* (2011) were able to show that the horse mackerel, *Megalaspis cordyla* from four areas, two each from the east (Digha and Mandapam regions in the Bay of Bengal) and west (Cochin and Mumbai regions of the Arabian sea) coasts of the Indian peninsula belonged to separate spawning stock populations. Similarly, Sen *et al.* (2011) studied the stock structure of *Decapterus russelli* from east and west coasts of

India which revealed the existence of two morphologically different stocks. Jayasankar *et al.* (2004) who carried out morphometric and genetic analysis of the Indian mackerel (*Rastrelliger kanagurta*) could not find significant differences among the three populations from selected centres in the east and west coasts of India. Mohandas (1997) analysed the morphometric data of oil sardine populations collected from Cochin, Calicut, Mangalore and Mandapam regions and observed that the populations from Calicut and Mandapam appeared to be morphologically similar.

The oil sardine populations along Kochi and Chennai coasts show apparent visual differences in shape and form. The population from Chennai coast is characterized by their relatively large head and long and slender body shape whereas the population from Kochi has a sub-cylindrical body with a round belly. At first glance they look like different stocks, and therefore, a study was undertaken using truss morphometric analysis to find out if the two populations belonges to the same stock or not. The feeding conditions and length-weight relationship of the sardines from Kochi and Chennai was also assessed to find out potential differences in these biological characteristics.

Material and methods

Sample collection

Two hundred adult specimens of oil sardine *S. longiceps* with total length ranging from 16 to 19 cm were collected from two locations viz., Chennai in the southeast coast and Kochi in the southwest coast of India (Fig. 1) during February-November 2011 from catches of ringseines and gillnets. The collected specimens were placed in insulated box with ice packs and brought to the laboratory for analysis.

Truss morphometrics

The specimens were placed on a water resistant paper and the body and fins were teased into a natural position to identify the landmarks. In the present study, the truss protocol of oil sardine was constructed based on 10 homologous anatomical landmarks (Table 1) and the truss network was constructed by interconnecting the landmarks to form a total of 21 truss measurements (Fig. 2) using paper and pin method (Strauss and Fuiman, 1985)

These distances were based on morphologically significant anatomical locations or points called 'morphometric landmarks'. Morphometric landmarks are true homologous points identified by some consistent feature of the local morphology (Jardine, 1969; Schaeffer, 1976; Strauss and

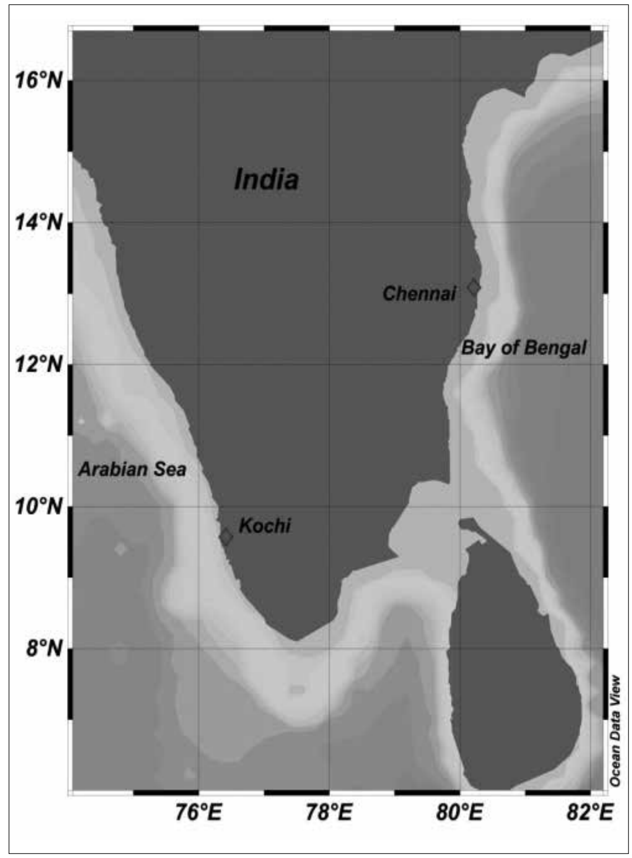

Fig. 1. Map showing sampling locations

Table 1. Landmarks used for extracting truss measurements from *S.longiceps*

Landmark No	Landmark position
1	Anterior tip of snout on upper jaw
2	Insertion of preopercle below anterior margin of eye
3	Nape above insertion of opercle
4	Origin of pectoral fin
5	Origin of first dorsal fin
6	Origin of anal fin
7	Insertion of dorsal fin
8	Insertion of anal fin
9	Dorsal origin of caudal fin
10	Ventral origin of caudal fin

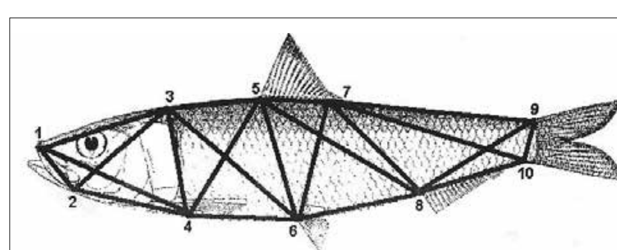

Fig. 2. Truss network of *Sardinella longiceps* showing the twenty one distances collated from 10 landmarks

Bookstein, 1982). At the point of the landmark, a hole was made and marked on the water resistant paper, using a dissecting needle. These points were then transferred to a graph sheet and the X-Y coordinate data were extracted to measure the truss distance between pairs of landmarks using the Pythagorean theorem.

Transformation for removing size dependent effects

As significant correlations were observed between body size and truss distances, the size dependent variation in the whole data may discriminate the stocks (Humphries *et al.*, 1981). Hence, a transformation of the absolute truss distances into size dependent shape variables was carried out. First, the outliers were removed based on Cook's distance estimates using PROC ROBUSTREG procedure of SAS (SAS Institute, 2011) to prevent the distortion of general tendency in the size distribution. A total of 120 measurements were selected after removing the outliers. Further, the size dependent effects were removed using an allometric approach by modifying the formula provided by Ihsen *et al.* (1981) and Hurlbut and Clay (1998). Data were transformed using the formula:

$$M_{adj} = M\,(SL_{mean} / SL)^{ß}$$

Where,

M_{adj} = transformed morphometric measurement
M = original morphometric measurement
SL = standard length of fish
SL_{mean} = overall mean standard length of the fish
ß = within group slope of the linear regression between log transformed M and log transformed SL.

Multivariate analysis

The Mardia's test was carried out to check the multivariate normality in the transformed truss distance data (Cox and Small, 1978). PROC MODEL procedure in SAS (SAS Institute, 2011) was used to carry out the Mardia's test for multivariate normal distribution.

Further, the distances were subjected to Factor Analysis (FA) using the PROC FACTOR (SAS Institute, 2011) procedure of SAS (Hatcher, 2003) to find out differences between the samples from the two localities. A maximum likelihood method was used to extract the factors. The retained factors were subjected to varimax (orthogonal) rotation procedure. For identifying the variables that demonstrate high loadings for a given component, the rotated factors were subjected to scratching procedure as described by Hatcher (2003).

Diet analysis

Freshly collected fish samples from southeast and southwest coasts were transported to the laboratory in ice and individual fish were evaluated for the following: Total length (mm), total weight (g), maturity stage (immature, maturing, ripe and spent) and stomach fullness (empty, traces to 1/4 full, 1/2 full, 3/4 full or full). To study the variations in food intake, individual fish were cut open and depending on the state of distension of the stomach were assigned as poorly fed (empty to 1/4 full), moderate (1/2 full) and actively fed (3/4 to full).To obtain information on the seasonal diet variations, data were analysed according to seasons based on ecological characters (Menon *et al.*, 2000) and classified as follows - Pre-monsoon (February to May), Monsoon (June to September) and Post-monsoon (October to January).

Length weight relationship

Total length and weight were recorded to the nearest 1mm and 1 mg respectively. Sex was determined by macroscopic examination of the gonads. The method suggested by Le Cren (1951) was followed to compute the length and weight relationship. The length-weight relationship was expressed as: $W = a\,L^{b}$ where W and L are weight (g) and length (cm) of the fish respectively and 'a' and 'b' are constants (initial growth index and regression constants respectively). When expressed logarithmically, the above equation becomes a straight line of the formula: $\text{Log } W = \log a + b \log L$. This linear equation was fitted separately for males, females and pooled samples. Regression analysis was performed to determine the constants a and b and relationship between total length and weight using the Data Analysis package in MICROSOFT EXCEL. The correlation coefficient (r) was determined to know the strength and pattern of association between the two variables.

The regression coefficients of sexes were compared by the analysis of covariance (ANCOVA) to establish the variations in the 'b' values, if any, between them. The significance of difference, in the estimate of 'b' in males , females and pooled data of sexes from the expected value of 3 (isometric growth) was tested by Bailey's t-test (Snedecor and Cochran, 1967) following the formula, $t = b\text{-}3/Sb$, where,

b = regression coefficient of log transformed data and
Sb = standard error of b

Condition factor K, a measure of the well-being or plumpness of a fish, was calculated following the equation proposed by Fulton (1904). It assumes that the standard weight of a fish is proportional to the cube of its length.

$K=100\ (W/L^3)$

where W is the weight of the fish in grams and L is the total length of the fish in mm. The factor 100 was used to bring the value of K close to 1.

Results and discussion

The factor analysis revealed that there is no significant morphometric variation between individuals obtained from southeast and southwest coasts and the variables with high loadings on the first three factors were not useful in distinguishing these samples. The result revealed that there is a single stock of oil sardine existing along the southeast and southwest coasts of India.

Differentiation of population between southeast and southwest coast of India

The first three factors explained 85.33% of the total variation in the data; with first, second and third factors contributing 49.95%, 19.17% and 16.21% of the variation respectively. The variables 1-2, 1-4, 2-3, 3-4, 3-6, 4-5, 5-6, 5-8 and 6-7 had the highest loadings on the first factor. These factors were concentrated on the anterior and middle portions of the fish body. The variables 3-5, 4-6, 5-7, 6-8, 7-8, and 7-10 loaded on second factor were related to pectoral, dorsal, anal and caudal fin regions. The factor 3 was loaded heavily with truss variables 8-9 and 9-10 which were related to the region between the insertion of anal fin and dorsal and ventral origin of caudal fin.

However, none of the factors have shown difference in the samples from southeast and southwest coast. Moreover, bivariate score plots between three factors revealed great degree of morphological homogeneity between oil sardine populations from Chennai and Kochi regions (Fig. 3a, b, c).

The truss morphometric analysis has revealed phenotypic homogeneity among the populations along the southeast and southwest coasts of India. The identification of distinct populations or stocks which are geographically or temporarily isolated from one another forms one of the important aspects of fisheries management (Booke, 1981). But, in the case of oil sardine, the migratory behavior of the species gives more chance for intermixing of stocks and therefore no reproductive isolation or separation of spawning grounds was observed which are important factors regarding stock separation (Hedgecock *et al.,* 1989; DFO 2004; Demer *et al.,* 2012., Izzo *et al.,* 2012 and Kumar *et al.,* 2012).

The phenotypic differences in oil sardine between the two coasts may be due to differences in the feeding habit

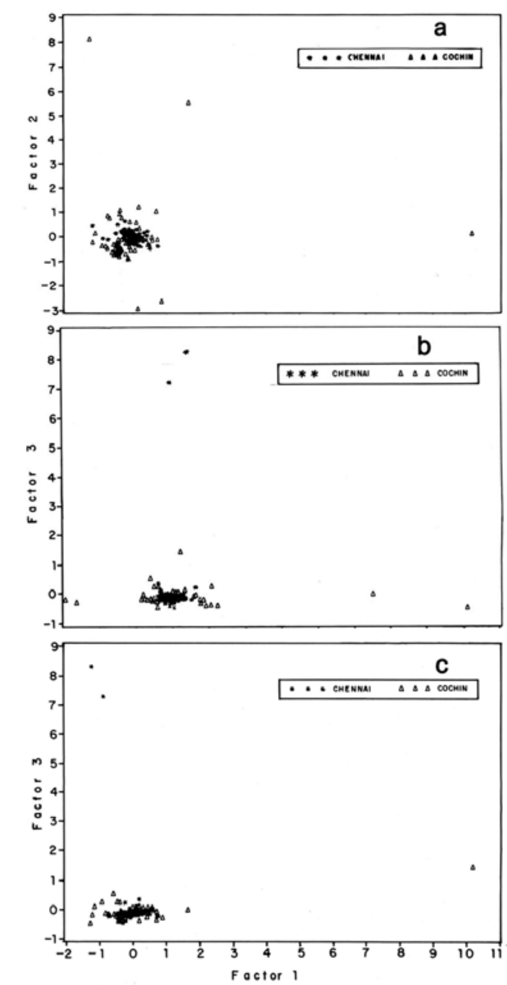

Fig. 3. Comparison plots of factor scores a) first and second b) second and third c) first and third from truss distances of *S. longiceps*.

between the two coasts (Remya *et al.*, 2013), but the differences have not been reflected as heterogeneity of the stock. Feeding intensity study along the Kochi coast showed predominantly moderate feeding activity during pre-monsoon season followed by poor feeding activity during monsoon and post-monsoon seasons for adults. For juveniles, moderate feeding activity during pre-monsoon, active feeding during monsoon and poor feeding activity during post-monsoon season was observed (Fig.4). On the contrary, poor feeding activity was observed in all the seasons for both adults and juveniles of oil sardine along Chennai coast (Fig.5). The low productivity of the Bay of Bengal ecosystem appears to be reflected in the feeding intensity of oil sardine along Chennai coast, and this may be the likely cause of slender shape. Site specific feeding studies in earlier reports also prove the same result (Sreekanth *et al.*, 2012). However, further studies are needed to confirm this.

The results of length-weight relationship of males and females of oil sardine from Kochi and Chennai obtained by logarithmic regression equations and their corresponding parabolic equations are given in Table 2. The analysis indicates positive allometric growth of oil sardine along Kochi coast and negative allometric growth along Chennai coast. The value of regression coefficient 'b' was found to be 3.1, and 3.2 for males and females of oil sardine along Kochi coast and 2.7 and 2.2 males and females along Chennai coast. When b=3, increase in weight is isometric i.e., length increases in equal proportion with body weight. When the value of b is varies from 3, weight increase is said to be allometric (positive if b>3 and negative if b<3). The 'b' value above 3 indicates that the fish become wider or deeper as they grow, while an exponent below 3 indicates they become more slender. In the present investigation, along Kochi coast, 'b' is greater than 3, indicating that the fish shape is becoming deeper as the length increases, and along Chennai coast, 'b' is less than 3, indicating that the fish is more slender with increase in length.

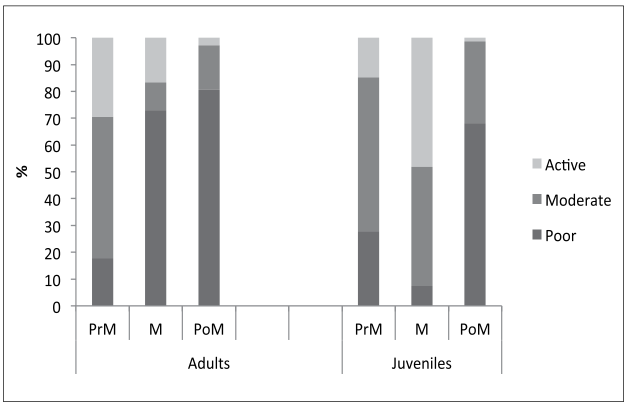

Fig. 4. Feeding intensity of adults and juveniles of oil sardine from Kochi coast during pre-monsoon, monsoon and post monsoon

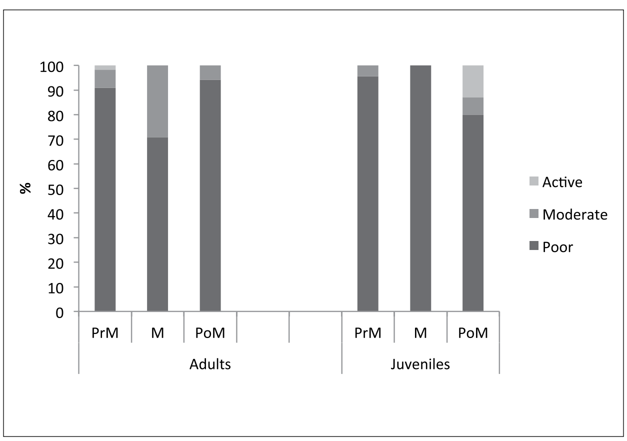

Fig. 5. Feeding intensity of adults and juveniles of oil sardine from Chennai coast during pre-monsoon, monsoon and post-monsoon

The regression equations between male and female oil sardine from Kochi and Chennai coasts were tested for equality through (ANCOVA) are shown in Table 3. The table shows that the values of slope from both the coasts do not differ significantly at 5% level. Therefore, a common equation for the species was calculated after pooling data of males and females of both the coasts. The significance of variation in the estimates of regression coefficient value 'b' from '3' showed significant difference at 1% level (Table 4), from the expected value of 3 indicating allometric growth in the species from both the coasts.

Variations in a fish's condition factor (K) primarily reflect state of sexual maturity and degree of nourishment. Condition values may also vary with fish age, season and in some species, with sex (Sebastian, 2011). K factor varies with species and size, but larger values generally are indicative of better fish condition. The condition factor K for males, females and pooled was higher during monsoon when compared to pre-monsoon and post monsoon seasons with values 1.0542, 1.0452 and 1.0313 for males, females and pooled respectively along Kochi coast whereas along Chennai, the condition factor K did not show much variation from 0.7 in males, females and pooled during all seasons (Table 5). This analysis has indicated better condition of the fish along Kochi coast when compared to Chennai coast. All these factors point to the fact that, oil sardine off Kochi has a better growth rate than those off Chennai coast, probably due to the difference in ecological conditions between the two coasts.

It can be concluded that the oil sardine population is a single stock along the southeast and southwest coasts of India

Table 2. Length weight relationship of oil sardine samples from Kochi and Chennai

Centre	Sex	Linear relationship	Parabolic relationship	r^2
Kochi	Male	Log W= -5.0409+$^{3.1034}$ Log L	$W=0.0065\ L^{3.103}$	0.818
	Female	Log W=-5.2119+$^{3.1692}$ Log L	$W=0.0055\ L^{3.169}$	0.827
	Pooled	Log W=-5.1399+$^{3.1414}$ Log L	$W=0.0059\ L^{3.141}$	0.8227
Chennai	Male	Log W= -4.36663+$^{2.7997}$ Log L	$W=0.0126\ L^{2.799}$	0.864
	Female	Log W=-2.53157+$^{2.1562}$ Log L	$W=0.0795\ L^{2.126}$	0.845
	Pooled	Log W=-3.64569+$^{2.5458}$ Log L	$W=0.0261\ L^{2.545}$	0.849

Table 3. Comparison of length-weight regression lines between male, female and pooled oil sardine along Kochi and Chennai coasts by ANACOVA

Kochi	Regression coefficient	Deviation from regression			Fvalue	Significance
		Df	SS	MSS		
Male	3.103	772	25.502	0.033	0.795	P = 0.373 (Not significant at 5% level)
Female	3.168	899	33.241	0.037		
Total		1671	58.743	0.035		
Pooled	3.140	1672	58.770	0.035		
Difference		1	0.028	0.028		
Total		1673	58.826	0.035		
Between adjusted means		1	0.056	0.056		
Chennai						
Male	2.799	207	2.372	0.011	0.759	P = 0.38 (Not significant at 5% level)
Female	2.156	206	1.116	0.005		
Total		413	3.489	0.008		
Pooled	2.545	414	3.813	0.009		
Difference		1	0.323	0.323		
Total		415	3.820			
Between adjusted means		1	0.006	0.006		

Table 4.Bailey's 't' value showing comparison of regression coefficient 'b' for male, female and pooled oil sardine from Kochi and Chennai coasts.

Region	Gender	t value	Significant or not (at 1% level)
Kochi	Male	1.961	significant
	Female	3.460	significant
	Pooled	3.966	significant
Chennai	Male	-2.598	significant
	Female	-13.125	significant
	Pooled	-8.607	significant

Table 5. Condition factor (K) of oil sardine for male, female and pooled oil sardine along Kochi and Chennai coast

Region	Gender	Pre Monsoon	Monsoon	Post Monsoon
Kochi	Male	0.8095	1.0542	0.8759
	Female	0.7887	1.0452	0.8903
	Pooled	0.7887	1.0313	0.85
Chennai	Male	0.7286	0.7296	0.7527
	Female	0.6850	0.7139	0.7786
	Pooled	0.7067	0.7187	0.7682

with no significant morphometric variations. The reasons for marginal differences in shape and form are attributed to the ecological differences in the habitats. However, it is recommended that genotypic methods in addition to the present phenotypic study may also be carried out for confirmation of the present results.

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