Morphometric and genetic variations in the threadfin bream *Nemipterus mesoprion*

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Abstract

Truss network analysis and protein gel electrophoresis were used as tools to find out intraspecific differences between *Nemipterus mesoprion* populations collected from the marine landings of Chennai and Kochi. Truss morphometric studies showed morphologically homogenous populations. Marked differences in allele frequencies between the two populations were not evident in protein electrophoresis. Average heterozygosity values for Chennai and Kochi populations were 0.643 and 0.718 respectively. One out of the three loci in the populations showed significant deviations from Hardy - Weinberg equilibrium. Genetic identity and genetic distance values were 0.99962 and 0.0038 respectively. Electrophoretic results confirmed morphological conclusions.

An understanding of the population structure and intraspecific variation of fish is fundamental to fisheries management and effective conservation of genetic resources. Morphometric and anatomical measurements have traditionally been used to differentiate populations and fish stocks. Morphometric analysis has been applied to many stock differentiation and life-history problems in temperate species (Bronte *et al.*, 1999). If shape differences in different populations of the same species can be used to discriminate morphotypes, they may also be useful in examining the stock structure within a morphotype, they may also be useful in examining the stock structure within a morphotype. Detection of differences within a morphotype may indicate geographically separated stocks, whose shapes may be predicted on local environmental conditions or have genetic bases.

Truss morphometrics is a better tool than traditional methods alone for probing evolutionary processes or elucidating relationships among populations (Winans, 1984). Multivariate analysis of morphological measures was found useful to study intraspecific ecological diversification in Omul, *Coregonus autumnalis migratorius* (Bronte *et al.*, 1999). Sex identification in Nile tilapia was done based on truss morphometrics (Brzeski and Doyle, 1988) while Velasco *et al.* (1996) characterized eight strains of the same species using truss morphometrics.

Stock identification based on electrophoreticaly measured biochemical differences is currently used in fisheries management as a tool for estimating stock composition in mixed stock fisheries (Milner *et al.*, 1981). Threadfin breams form an important demersal fishery resource along Indian coasts and of late

they have been receiving increasing attention because of their use in the manufacture of surimi and surimi-based products. Few attempts have been made to study the genetic variation in nemipterids (Chakraborthy, 1989; Santos, 1993). In our study, truss morphometrics and protein gel electrophoresis were applied with a view to determining genetic difference, if any, between the samples of *Nempiterus mesoprion* from marine landings at Chennai and Kochi.

Material and methods

For Truss network analysis, 100 specimens of *N. mesoprion* each from Thoppumpady fishing harbour, Kochi and Kashimedu fishing harbour, Chennai were collected from commercial trawl catches during October, 1999-April, 2000. Total length and Weight ranges were 96-260 mm/96-230 g (Kochi) and 122-171 mm/ 22-53 g (Chennai). The Truss protocol (Strauss and Bookstein, 1982; Winans, 1984) was used to describe the shape of each fish through the specification of a set of morphometric characters. The truss is a system of vertical, horizontal and ob-

lique distances measured between preselected anatomical landmarks (Fig.1), which are points identified on the basis of local morphological features and chosen to divide the body into functional units (Bookstein *et al.*, 1985). Principal Component Analysis (PCA) was carried out on these network distances (Morrison, 1990). Further analysis of the clusters was done by the Sheared PCA (Humphries *et al.*, 1981).

For electrophoretic analysis, 14 numbers each of *N. mesoprion* samples were collected from Thoppumpady and Kashimedu fishing harbour. They were immediately transported to lab in iceboxes packed with crushed ice. Protein was extracted from white muscle using standard procedures. Extreme care was taken to separate red muscle while preparing tissue samples. Electrophoresis was carried out using PAGE (Laemmli, 1970) with slight modifications. Nomenclature of protein loci and allele designation follows Shaklee *et al.* (1990). Allele frequency, expected genotype frequency, heterozygosity, genetic identity and genetic distance were calculated (Nei, 1972, 1978).
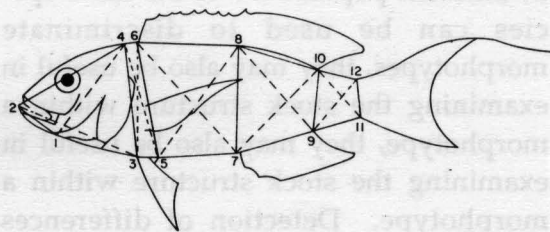

Fig. 1 Outline drawing of *Nempiterus mesoprion* showing the locations of the 12 landmark points and the morphometric distance measures recorded on each individual fish

Results and discussion

Principal Component Analysis (PCA) does not require any prior information about the groups in the analysis of truss data. The first component factor of PCA is interpreted as size component and subsequent factors are designated as shape variables. The percentage of variation explained by these factors should be considered before conclusions are made. In the present study, 26 truss measurements were made on samples from the two centres. Among the resultant 26 principal components, the first (PC-I) and the second (PC-II) principal components accounted for 78.1% of the total cumulative variation. XY scatter diagram obtained by plotting PC-I on X axis and PC-II on Y axis showed that the clusters were mixed up (Fig. 2A), indicating mixing of stocks from the two coasts. When sheared PCA was done, PC-I and PC-II accounted for

72.87% of the total cumulative variation. Plot of the sheared PC-I against the sheared PC-II showed no spearation (Fig. 2B), further indicating morphological homogeneity of the tested populations.

In electrophoretic analysis, three loci were identified as polymorphic out of the 9 loci analysed. Allele frequencies at these 3 loci did not show any marked differences (Table 1). While comparing observed and expected genotype frequencies in the two populations, it was observed that only locus 1 did not conform to Hardy-Weinberg equilibrium (Table 2).

Although electrophoretic data provide valuable information for evaluation of intraspecific genetic variablility, complementary data from other sources are needed for a comprehensive view of population differentiation. One of the reasons for the poor separation of stocks could be

Table 1. *Allele frequencies, Observed (Ho) and Expected (He) heterozygosity values, Genetic identity and Distance values at 3 loci in N. mesoprion populations from Kochi and Chennai*

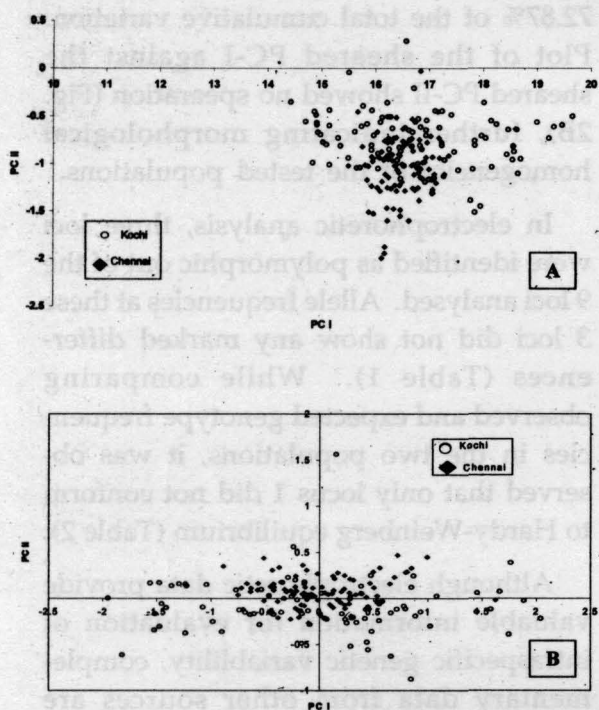| Locus	Allele	Kochi			Chennai			Genetic identity	Genetic Distance
		Allele Frequency	Ho	He	Allele Frequency	Ho	He		
1	97.22	0.50			0.46				
			1	0.50		0.93	0.50	0.998	0.002
	100	0.50			0.54				
2	100	0.69			0.68				
			0.62	0.43		0.64	0.44	0.999	0.001
	105.26	0.31			0.32				
3	100	0.73			0.82				
			0.54	0.40		0.36	0.29	0.991	0.009
	102.38	0.27			0.18				

Fig. 2 A *Principal Component Analysis of Truss network land marks of Nemipterus mesoprion from Kochi and Chennai*

B *Sheared Principal Component Analysis of Truss network land marks of Nemipterus mesoprion from Kochi and Chennai*

the number of samples used and the number of truss lengths measured (Velasco *et al.*, 1996). Misra and Ni (1983) suggested that using a large number of characters with limited samples can be inappropriate in discriminant analysis. However, it is suggested that in truss network analysis, if the number of individuals minus the number of variables measured is more than 30, then the sample size can be considered as adequate (Harris, 1975). Hence the sample size used in this study is adequate.

Both the electrophoretic studies and truss morphometric revealed that the 2 populations from Chennai and Kochi are homogenous. The fact that genetic identity values between the two populations at all the three loci were close to 1 (Table 1), lends support to this view. Average heterozygosity values of *N. mesoprion* from Chennai and Kochi (0.643 and 0.718, respectively) observed in the present study

Table 2. *Observed and expected genotype frequencies in N. mesoprion populations from Kochi and Chennai.*

Locus	Genotype	Kochi		Chennai	
		Observed (Expected)	X^2	Observed (Expected)	X^2
1.	97.22/97.22	0 (3.25)		0 (3.014)	
	97.22/100	13 (6.5)	13*	13 (6.96)	10.52*
	100/100	0 (3.25)		1 (4.022)	
2	100/100	5 (6.225)		5 (6.45)	
	100/105.26	8 (5.5415)	2.57	((6.10)	3.15
	105.26/105.26	0 (1.233)		0 (1.4425)	
3	100/100	6 (6.947)		9 (9.436)	
	100/102.38	7 (5.1126)	1.77	5 (4.115)	0.66
	102.38	0 (0.9406)		0 (0.448)	

*(p<0.05)

were higher than those reported in other marine fishes (Menezes and Parulekar, 1998). Based on the present data on heterozygosity and genotype frequencies, we opine that some form of heterogenous advantage, such as survival, adaptability or growth existed in the samples causing high heterozygosity.

The average D values obtained for the comparison of the two populations was 0.0038, which was well within the range (0-0.005), suggested for local races of a species (Taniguchi *et al.*, 1986).

The present study could not reveal any marked differentiation between two populations of *N. mesoprion* from Chennai and Kochi. Low levels of genetic divergence have been detected among marine fishes (Ward *et al.*, 1994). The relative lack of physical barriers and high incidence of excessive larval dispersal in marine systems generally result in little intraspecific genetic divergence, even over considerable geographic distances (Gyllensten, 1985). However, it is suggested to ratify the present results using DNA-level markers, such as RAPD, AFLP or micro satellites, which can provide greater number of polymorphic markers.

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