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Stock discrimination of vermiculated spinefoot, *Siganus vermiculatus* (Valenciennes, 1835), from the South Konkan coast of Maharashtra using truss morphometry

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Original Article

Abstract

Vermiculated spinefoot, *Siganus vermiculatus* is one of the fastest growing and commercially important siganid species occurring in Indian waters. Stock discrimination of vermiculated spinefoot was carried out along the south Konkan coast of Maharashtra by using truss morphometry. The present investigation was based on a study of 66 individuals collected from Harnai, Ratnagiri and Malvan locations of the South Konkan coast of Maharashtra. A 14-point truss network with 26 truss variables was studied. Truss morphometry showed significant differences in II (4-5), MM (5-6), NN (5-10), OO (5-11), SS (6-10), TT (6-11), XX (10-11), YY (11-12) and ZZ (12-14) distances from the three sampling locations in 26 truss morphometric measurements thereby indicating phenotypic heterogeneity among populations of *Siganus vermiculatus* from the south Konkan coast at a small spatial resolution.

Keywords: *Stock discrimination, Siganus vermiculatus, South Konkan*

Introduction

The Siganids, commonly known as rabbit fish or spinefoot are medium sized, herbivorous fishes belonging to the family Siganidae. They comprised 13 species belonging to the genus *Siganus* (Fischer and Bianchi, 1984). Siganids are distributed in reefs, sea grasses, mangroves and estuaries of tropical and subtropical coastal environment (Woodland, 1997). *Siganus canaliculatus, S. javus, S. lineatus, S. stellatus* and *S. vermiculatus* are important spinefoot species found in India (Anand and Reddy, 2012). *S. canaliculatus, S. vermiculatus* and *S. javus* commonly occur along the Ratnagiri coast. Siganids resemble each other in most of the features. They are identified by their deep, compressed body, snout resembling that of a rabbit, 13 strong spines in the dorsal fin, 7 spines in the anal fin, and 2 spines in the ventral fin. The skin is leathery, and the scales are smooth, small and closely adherent. The fishes are often mistaken as without scales. The colour is olive- green to brown depending on the species (Herre and Montalban, 1928; Munro, 1967).

Siganids are ideal candidate species for aquaculture because of their rapid growth, herbivorous feeding habits, commercial value and tasty flesh (Lam, 1974). Vermiculated spinefoot, *S. vermiculatus* is one of the fastest-growing siganid species (Anuraj *et al*., 2021) and has been gaining commercial importance owing to its meat quality and high market potential (Kitche-Arreglado *et al*., 2013). The species is the most estuary-dependent of all siganids (Woodland, 1990). It is listed as least concern and monitoring of harvest levels as well as other potential threats is recommended (The IUCN Red List of Threatened Species, ISSN 2307-8235 (online) IUCN 2011 T196436A2457345). The stock structure analysis encompassing stock identification and population discrimination of a particular species plays a vital role in scientific resource management and stock enhancement programs (Shaklee and Bentzen, 1998). The information is necessary to obtain to achieve sustainable yield, avoid recruitment failures, rebuild the overfished stocks, and conserve threatened and endangered species. Morphological characteristics, such as body shape and meristic counts, have long been used to delineate stocks (Heincke, 1898), and continue to be used successfully (Villaluz and Maccrimmon, 1988; Haddon and Willis, 1995; Silva, 2003). Morphometric differences among stocks of a species are important for evaluating the population structure and as the basis for identifying stocks (Ihssen *et al*., 1981; Turan, 2004). The morphometric analysis provides information on phenotypical stocks, equivalent growth groups, mortality rates and reproductive rates (Booke, 1981).

The Truss Network System is for morphometric measurements (Strauss and Bookstein, 1982; Strauss, 1985; Cardin and Friedland, 1999) and consists of a series of distances calculated between landmarks that form a regular pattern of connected quadrilaterals or cells across the body form (Strauss and Bookstein, 1982). The truss network system is a landmark-based technique of geometric morphometrics, which has no restriction on the direction of variation and localization of shape changes and is very effective in capturing information about the shape of an organism (Cavalcanti *et al*., 1999). The landmarks chosen are homologous, representing the identical developmental feature among specimens, and are easily located anatomically (Winans, 1985; Bookstein, 1990 and Cadrin, 2005). Truss Network Analysis is being increasingly used for the purpose of stock identification and discrimination. Studies on stock discrimination of various species by using truss morphometry have been variously reported by authors including Gopikrishna *et al.* (2006) for *Lates calcarifer*, Pawase (2010) for *Lactarius lactarius*, Swatipriyanka *et al.* (2011) for *Decapterus russeli*, Sajina *et al.* (2011) for *Megalaspsis cordyla*, Gorospe and Demayo (2013) for *Siganus guttatus*, Remya *et al.* (2014) for *Rastrelliger kanagurta*, Pawar *et al.* (2011); Hakim *et al.* (2019) for *Nemipterus japonicus*, Pazhymodon *et al.* (2015) for *Harpodon nehereus*, Rawat *et al.* (2019) for *Eubleekeria splendens*, Ahamad *et al.* (2003), etc.

In India, the brackish water fisheries are data deficient and are plagued with complex challenges attributed to habitat degradation, overexploitation, stock depletion, illegal fishing and climate change. Delineating the stocks of *S. vermiculatus* from the south Konkan coast of Maharashtra based on truss morphometry will pave the way for formulating management plans for the species in the future.

Material and methods

The study was carried out in two coastal districts of Maharashtra namely Ratnagiri and Sindhudurg representing the south Konkan coast. Geographically the study area is located between 17° 02′ 43″ E latitude and 73° 16′ 57″ E longitudes to 15° 43′ 46″ E latitude and 73° 40′ 37″ E longitudes (Fig. 1). The south Konkan coast has a coastline of 281 km and a continental shelf area of 52000 km^2. Sampling was carried out from three landing centres, *viz*., Harnai, Ratnagiri, and Malvan of south Konkan, which are situated along the Anjarle, Mirya, and Sarjekot estuaries.

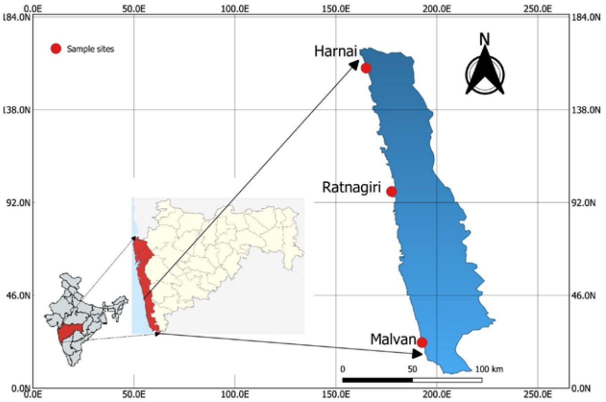

Fig. 1. Location of sample collaction points

Sample collection

A total of 66 specimens of *S. vermiculatus* ranging in size from 14.7 cm to 24.4 cm constituted the sample size for the study. Twenty-two samples were collected from each landing centre. The samples comprised of pooled individuals. No sexual dimorphism was observed in the collected individuals. The individuals were placed in an insulated ice box and brought to the laboratory. The samples were cleaned thoroughly in running water to remove the slime or dirt and stored temporarily in the freezer at –20 °C. The frozen samples were thawed adequately before further analysis.

Digitization of samples

Each fish was placed on a thick graph paper on its left side and assigned a specific code for identification. Digital photographs of each specimen were taken with Sony Cybershot DSC- W810 Point- and -shoot camera (image resolution 20.1 megapixels) (Fig. 2). Images of *S. vermiculatus* were digitized in CorelDraw (2021) version 23.0.0.363 software by dividing the individual image into six equal columns with the help of grid scale. Digitized images were used to record the landmarks.

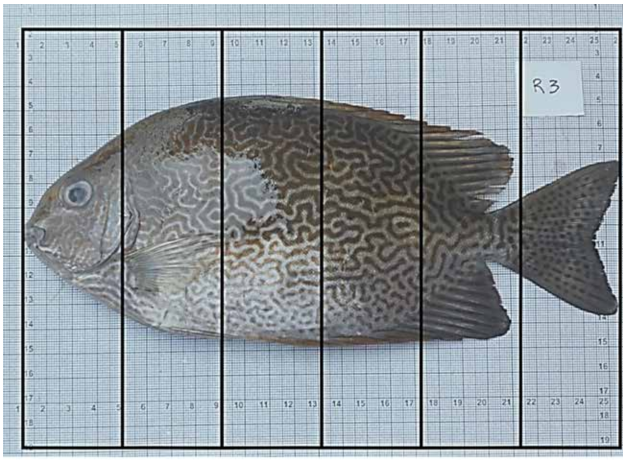

Fig. 2. *S. vermiculatus* on a flat platform with graph paper labelled with a specific code

Extracting truss morphometric

The landmarks used for extracting truss measurements from the body are given in (Fig. 4). The truss network was developed by interconnecting 14 landmarks leading to 26 truss measurements from each individual (Fig. 3). The truss morphometric data was extracted from each digitized image of the specimen by a combination of three softwares *viz*., tpsUtil V1.69 (Rohlf, 2015), tpsDig2 V2.26 (Rohlf, 2015) and Paleontological statistics (PAST) (Hammer *et al*., 2001).

All the images were first converted from JPEG (*. jpeg) to TPS (*. tps) format by using a utility program called tpsUtil V1.69 (Rohlf, 2015) and ordered into a single file. The input of the image in tps format is a prerequisite for the tpsDig2 programme to analyse and extract the morphometric data. The landmarks were digitized on the image using the 'digitized landmark' mode of the software, and the landmark data was encrypted into the tps files X-Y coordinates. The data-encrypted tps format image files were used as an input in the PAST. The data on distances between the landmarks were extracted using the 'all distances from landmark' and '2-dimensional' options of the 'Geomet' menu.

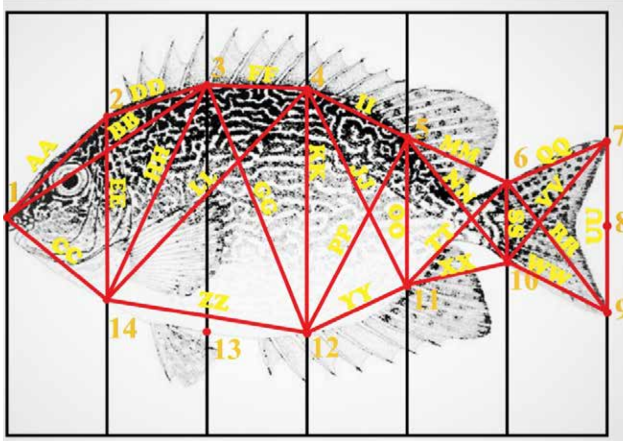

Fig. 3. Truss Network of *S. vermiculatus*

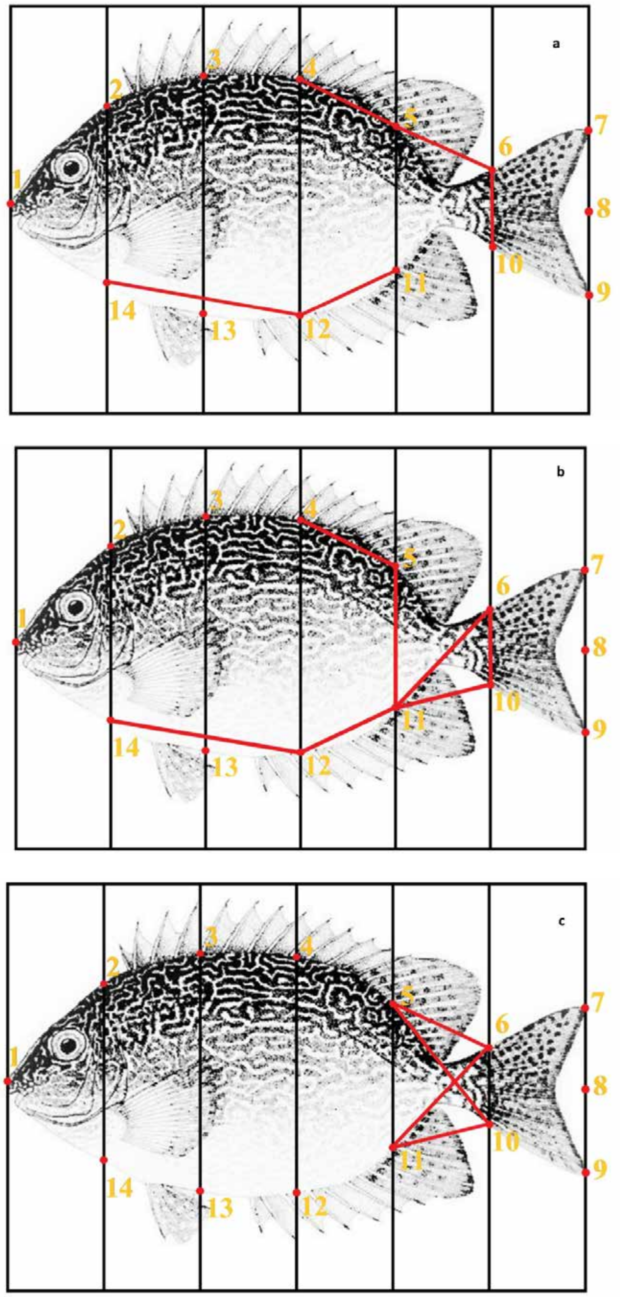

Fig 4. Truss morphometric differences among stocks, a) Ratnagiri –Harnai, b) Ratnagiri – Malvan stocks, c) Harnai -Malvan stocks

Statistical analysis of truss morphometry data

Multivariate analysis of variance (MANOVA) and classification accuracy were used in the statistical analysis of truss morphometric data. Prior to MANOVA, the data were standardized by transforming each measurement to a proportion of the total length of the individual to remove the bias of size differences and make inter-landmark measurements directly comparable among individuals (Canty *et al*., 2018). MANOVA was performed for 26 truss morphometric measurements to test the significant differences at different locations using SAS ver. 9.3. The classification accuracy was evaluated based on a percentage of individuals correctly assigned to the original sampling location and then calculating the proportion of correctly allocated individuals.

Results

Truss morphometry data

Multivariate analysis of variance: A total of 14 landmarks to get 26 standardized data morphometric measurements have been used for truss network analysis in the present study. Twenty- six standardized data truss morphometric measurements and their column-wise description are given in Table 1. Wilk's Lambda and Pillai's Trace showed a p-value of less than 0.05, demonstrating a difference between the locations. Among the truss morphometric measurements, the nine measurements *i.e.* II (4-5), MM (5-6), NN (5- 148 10), OO (5-11), SS (6-10), TT (6-11), XX (10-11), YY (11-12) and ZZ (12-14) were significantly different ($p < 0.05$) from the three sampling locations. However, the 17 other truss morphometric traits were not significantly different ($P < 0.05$) among the stocks at three sampling locations. The comparison between Ratnagiri and Harnai populations showed a significant difference ($p<0.05$) in the seven truss morphometric measurements, namely II (4-5), YY (11-12), SS (6-10), ZZ (12-14), OO (5-11), TT (6-11) and XX (10- 11) (Fig. 4a). The comparison between Ratnagiri and Malvan populations showed a significant difference ($p<0.05$) in the five truss morphometric measurements, namely II (4-5),

Table 1. MANOVA for standardized data on truss morphometric measurements (different superscripts indicate significant differences based on MANOVA followed by Tukey's HSD test; p <0.05)

			Sampling locations			MANOVA			
No	Landmarks	Distance	Harnai	Ratnagiri	Malvan	Wilks' Lambda		Pillai's Trace	
				Mean		F ratio	p- value	F ratio	p- value
1	1-2	AA	0.247^{a}	0.241^{a}	0.246^{a}				
2	1-3	BB	0.402^{a}	0.402^{a}	0.402^{a}				
3	1-14	CC	0.218^{a}	0.221^{a}	0.219^{a}				
4	2-3	DD	0.175^{a}	0.177^{a}	0.176^{a}				
5	2-14	EE	0.319^{a}	0.315^{a}	0.317^{a}				
6	3-4	FF	0.171^{a}	0.169^{a}	0.171^{a}				
7	3-12	GG	0.438^{a}	0.431^{a}	0.433^{a}				
8	3-14	HH	0.398^{a}	0.399^{a}	0.398^{a}				
9	4-5	II	0.193^{a}	0.188^{b}	0.192^{c}				
10	4-11	JJ	0.363^{a}	0.369^{a}	0.363^{a}				
11	4-12	KK	0.391^{a}	0.387^{a}	0.389^{a}				
12	4-14	LL	0.482^{a}	0.486^{a}	0.484^{a}				
13	5-6	MM	0.179^{a}	0.18^{b}	0.176^{c}				
14	5-10	NN	0.255^{b}	0.249^{a}	0.246^{b}	3.147	< 0.05	3.096	< 0.05
15	5-11	OO	0.234^{b}	0.248^{b}	0.236^{a}				
16	5-12	PP	0.346^{a}	0.35^{a}	0.345^{a}				
17	6-7	QQ	0.176^{a}	0.179^{a}	0.177^{a}				
18	6-9	RR	0.241^{a}	0.235^{a}	0.232^{a}				
19	6-10	SS	0.135^{a}	0.125^{b}	0.136^{c}				
20	6-11	TT	0.244^{a}	0.251^{b}	0.256^{c}				
21	7-9	UU	0.217^{a}	0.221^{a}	0.214^{a}				
22	7-10	VV	0.245^{a}	0.246^{a}	0.252^{a}				
23	9-10	WW	0.173^{a}	0.173^{a}	0.172^{a}				
24	10-11	XX	0.176^{a}	0.182^{b}	0.179^{c}				
25	11-12	YY	0.183^{a}	0.179^{b}	0.182^{c}				

YY (11-12), SS (6-10), ZZ (12-14) and MM (5-6) (Fig. 4b). The comparison between Harnai and Malvan populations showed a significant difference ($p<0.05$) in four truss morphometric measurements namely MM (5-6), NN (5-10), TT (6-11) and XX (10- 11) (Fig. 4c).

Classification accuracy

The results of group classification of individuals of Harnai, Ratnagiri and Malvan showed that 95.45%, 90.91% and 95.45% respectively were correctly classified.

Discussion

Truss morphometry data

Multivariate analysis of variance: Winans (1987) discovered that almost all of the morphometric characters sampled from finfish from the 1960s to the 1980s were based on those selected by Hubbs and Lagler (1947), which were primarily longitudinally oriented and were focused mainly on the head and tail regions of the fishes. As an alternative, Strauss and Bookstein (1982) proposed a technique of obtaining linear distances across the body surface of fish by creating a box-truss network between landmarks covering the entire body. Several researchers compared the overall performance of traditionally measured finfish dimensions to such box-truss distances and have observed that trussed data resulted in more accurate classification of individuals (Strauss and Bookstein, 1982; Winans, 1987; Schweigert, 1990; Roby *et al*., 1991). The Ratnagiri: Harnai populations differ significantly ($p<0.05$) in seven measurements namely II (4-5), YY (11-12), SS (6-10), ZZ (12-14), OO (5-11), TT (6- 11) and XX (10-11). II (4-5) and YY (11-12) represent the entire dorsal and ventral sections of the body adjacent to the caudal peduncle. Distance SS (6-10) represent the end of the caudal peduncle. ZZ (12-14) represents the ventral part of the body nearer to the snout. OO (5-11) represents the vertical distance on the posterior side of the body. TT (6-11) represents the region between the caudal peduncle and the middle of the anal fin, and XX (10-11) represents the lower part of the caudal peduncle. Comparisons between Ratnagiri and Malvan populations showed significant differences ($p<0.05$) in the five truss measurements namely II (4-5), YY (11-12), SS (6-10), ZZ (12-14) and MM (5-6). II (4- 5) and YY (11-12) represented the entire dorsal and ventral section of the body nearer to the caudal peduncle. Briefly, distances SS (6-10) represent the end of the caudal peduncle. ZZ (12-14) represents the ventral part of the body nearer to the snout, and MM (5-6) represents the upper part of the caudal peduncle. Comparisons between Harnai and Malvan populations showed significant differences ($p<0.05$) in four truss morphometric measurements, namely MM (5-6), NN (5-10), TT (6-11) and XX (10-11). Distance M (5-6) represents the upper part of the caudal peduncle. NN (5-10) represents the caudal region, TT (6-11) represents the region between the caudal peduncle and the middle of the anal fin, and XX (10-11) represents the lower part of the caudal peduncle. Stocks are historically discriminated based on phenotypic variation in life-history, meristic, morphometric and life-history traits. These characteristics are quantitative genetic traits controlled by many genes and affected by the environment in which those genes are expressed (Falconer, 1981; Hard, 1995). They are also related to fitness and moulded by natural selection, reflecting local adaptation (Carvalho, 1993; Hard, 1995; Conover, 1998). Thus, phenotypic differences observed in certain areas (truss distances) may reflect genetic differentiation, environmental differences or a combination of the two (Thompson, 1991). It would be difficult to pinpoint which of these factors is responsible for the differences found in the present study, given a general lack of information in this regard.

Differences in morphometric characters might be related to several environmental variables which influence the fish morphology, including diet (Wimberger, 1992; Tonn *et al.,* 1994; Olsson *et al.,* 2006; Cadrin *et al*., 2014), water temperature (Lõhmus *et al.,* 2010), predation pressure Scharnweber *et al*., 2013), habitat structure (Willis *et al*., 2005), depth (Mwanja *et al*., 2011) and water currents (Franssen *et al.,* 2013). Local hydrology can also be a driving force of morphometric differences as variations in environmental and behavioural factors can be reflected in changes in body forms and shapes (Webb, 1984). The causes of truss morphological variations across locations are sometimes difficult to explain, although it is widely known that morphometric characters may respond to environmental circumstances with a high degree of plasticity (Wimberger, 1992). For example, ecological interactions such as competition for food, space, and shelter, predation pressure, and hydrobiological factors such as water temperature and salinity (Rawat *et al*., 2019). Ecological and evolutionary processes cause changes in the morphological characteristics of the fish population. Variation in populations refers to differences in behavioural, morphology, or life cycle characteristics, and it is most often observed in vertebrate populations (Robinson and Wilson, 1994; Wimberger, 1994; Smith and Skulason, 1996).

In the present study, the populations of *S. vermiculatus* were sampled from the three locations at an approximate distance of 198 km from each other. It is unlikely that ecological interactions and hydrobiological parameters factors, including temperature and salinity, differ significantly within this limited range. The results thus prove the efficiency or robustness of truss morphometrics in discriminating populations at small spatial scales and short geographic distributional ranges. The applicability and efficiency of truss analysis in delineating fish populations at a smaller spatial scale are in agreement with Canty *et al.* (2018).

Classification accuracy

The classification accuracies for Harnai, Ratnagiri, and Malvan were 95.45%, 90.91%, and 95.45%, respectively. The high percentage of the wrong classifications in the present study was from the Ratnagiri location. The percentages of correct classification recorded were highest in the Harne and Malvan stock. In a way, this further validates the usefulness of truss morphometrics in stock discrimination.

Conclusion

Truss morphometry has been proven to be an accurate and robust tool for discriminating stocks at a shorter spatial scale as evidenced in the present study. This tool can inform evidence-based management of fishery resources thereby achieving sustainable fisheries. However, the genetic variation among the stocks of *Siganus vermiculatus* can also be examined using molecular genetic techniques such as mitochondrial DNA analysis.

Author contributions

Conceptualization: PJK; Methodology: VHN; Data Collection: PJK, SYM, SSG; Data analysis: RAP, SSG; Writing Original Draft: PJK; Writing Review and Editing: VHN, SYM; Supervision: PJK

Data availability

The data are available and can be requested from the corresponding author.

Conflict of interests

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Ethical statement

No ethical approval is required as the study does not include activities that require ethical approval or involve protected organisms/ human subjects/ collection of samples/ protected environments.

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