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Population delineation of mudskipper *Boleophthalmus dussumieri* (Valenciennes, 1837) from Indian waters

Pritam Das, Shashi Bhushan*, B. B. Nayak, P. Layana and K.V. Rajendran
ICAR-Central Institute of Fisheries Education, Versova, Mumbai - 400 061, Maharashtra, India

Abstract

The mudskipper *Boleophthalmus dussumieri* (Valenciennes, 1837) is an amphibious goby inhabiting muddy substrates, predominantly within mangrove ecosystems influenced by tidal fluctuations. In this study, a total of 292 specimens collected from Gujarat, Maharashtra and West Bengal were examined for stock differentiation using truss morphometry and parasites as biological tags. Twenty-six morphometric variables extracted from 12 anatomical landmarks were were anlysed using principal component analysis (PCA), which accounted for more than 80% of the total variation in the first two components. Linear discriminant analysis (LDA) indicated 100% correct classification, confirming the existence of three distinct stocks, with the leasr divergence observed between Maharashtra and Gujarat populations and the greatest divergence between Maharashtra and West Bengal populations. The only parasite identified, *Ellipsomyxa* sp., was confined to the gall bladder, but lacked the diagnostic features necessary to serve as a tag parasite for stock discrimination. Consequently, it was considered unsuitable for differentiating *B. dussumieri* populations.

*Correspondence e-mail:
Shashi@cife.edu.in

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Introduction

Boleophthalmus dussumieri (Valenciennes, 1837), known as mudskippers, are amphibious gobies that are fully terrestrial for some part of the life cycle (Murdy, 1989). Along with other members of the sub-family Oxudercinae, this species forms a diverse group that has colonised semi-terrestrial habitats in intertidal and supratidal coastal swamps (Polgar *et al*., 2009). The sub-family Oxudercinae, comprises 10 genera and 40 species, distributed across tropical and subtropical regions of the Indo-west pacific (Murdy, 1989). Although distributed all along the Indian coast, *B. dussumieri* is most abundant along the north-west coast (Maharashtra and Gujarat) and the north-east coast (Sundarban region) of India. (Shukla *et al*., 2014; Mahadevan and Ravi, 2015). *B. dussumieri* holds traditional importance as a source of food, medicine and as a biological indicator of pollution, providing valuable insights into the health of the mangrove ecosystem. Though this species does not support a major fishery in India, it has been a valuable source of food and medicine in some selected regions of the country, which supports the need for detailed knowledge on the stock structure and distribution to guide effective resource management.

The concept of the stock is fundamental to fisheries management and refers to a subset of a species characterised by shared growth and mortality parameters within a defined geographical area (Vivekanandan, 2005). Modern stock identification studies often integrate genetic and phenotypic data to delineate the number and boundaries of non-inter-breeding, self-recruiting populations within an exploited species (Ovenden, 1990). The broad distribution, dynamic abundance and temporal variability of many fish species pose significant challenges for management, as these factors are difficult to observe directly. Effective exploitation, management and sustainable development of a fishery therefore require precise information stock structure and identity to maintain productivity and maximise yield (Lorenzen, 2008).

Among the various methods of stock identification, morphological characters have traditionally served as the primary basis for classifying organisms and distinguishing fish stocks. Similarities and differences in these traits are used to assess relationships, with closely related groups showing greater morphological resemblance and more divergent groups exhibiting pronounced differences. Although this approach is longstanding, the integration of modern analytical techniques now enhances the precision of morphological assessments for fish identification and stock discrimination.

Truss network system or truss morphometry is a landmark-based technique where fishes are placed on a geometric graph paper for digitisation of fish and provide no restriction on the direction of variation or in shape changes. The truss network system is highly effective in capturing information about the shape of an organism (Cavalcanti *et al*., 1999). It calculates a distance series based on the landmark selected from the digitised photo forming quadrilaterals or cells across the body form (Strauss and Bookstein, 1982). Many researchers have found the box-truss network method to be more effective than traditional morphometric approaches for identifying stocks or detecting intra-specific variations (Strauss and Bookstein, 1982). However, a key information is that morphologically different stocks may share the same genetic background. Environmentally induced phenotypic variation can be advantageous for stock identification, especially when the time is limited for significant genetic differentiation to accumulate among populations. Due to random genetic drift, genetic differentiation may occur very slowly in the typically large populations of commercial marine fishes (Ward *et al*., 1994). Nevertheless, numerous morphometric studies have provided evidence of stock discreteness, including the work on *Cyprinus carpio* (Corti *et al*., 1988), *Hoplostethus atlanticus* (Haddon *et al*., 1995), *Engraulisen crasicolus* (Bembo *et al*., 1996).

The use of parasite for identification of fish stock is a well-known approach in population studies. Parasites are naturally acquired by their fish host and can provide valuable ecological information on nursery grounds, migration patterns and foraging history (Thomas *et al*., 1996). Depending on the environmental parameters, a parasite's life span within a fish host may range from a few months to more than a year. Selecting a suitable tag parasite requires prior knowledge form earlier studies or a comprehensive survey of the parasite community in the target fish species. Ideal tag parasites should exhibit characteristics such as low mortality rate, ease of preservation and identification, sufficient longevity within the host, variation in abundance across different eco-regions and high host specificity (MacKenzie and Abaunza, 1998). In parasitological studies, it is essential to assess community diversity (number of parasite species recovered), prevalence (percentage of infected fish), abundance (average number of parasites per fish host) and intensity (average number of parasite per infected fish host). Fish stock discrimination based on parasite tag has been documented in several species like, *Katsuwonus pelamis* (Lester *et al*., 1985), *Istiophorus platypterus* (Speare, 1995) *Engraulis ringens* (Valdivia *et al*., 2007).

In the present study, linear distances between truss network landmarks were used to identify phenotypic stocks of the mudskipper *B. dussumieri,* along the Indian coast, based on patterns of morphometric variation and parasite distribution between fish populations.

Materials and methods

Sampling

During this study, samples of *B. dussumieri* were collected from Bhayandar Fish Market and Karanja Estuary (Maharashtra) and Navasari (Gujarat) on the west coast as well as from Junput and Kakdwip (West Bengal) on the east coast, during September 2019 to February 2020 (Fig. 1). Species identification of *B. dussumieri* followed the description by Murdy (1989).

Acquisition of morphometric data

A total of 292 specimens of *B. dussumieri* were collected during the present study for stock identification. Fishes were sampled taking care to avoid any physical injury. The collected specimens were transported in insulated fish boxes to the laboratory. Specimens were digitised for truss morphometric analyses, and the remaining

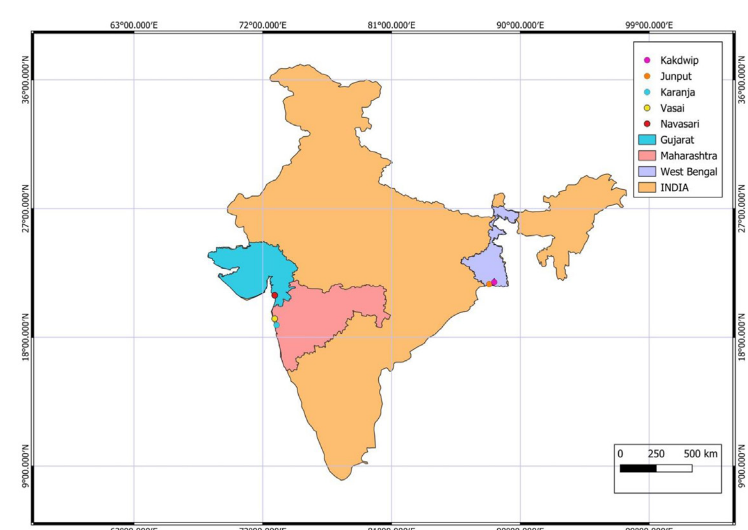

Fig. 1. Sampling locations of *B. dussumieri*

samples were stored at -20°C for further truss and related Frozen samples were later thawed in running water, dried and wiped for subsequent studies. Additional live specimens were also collected specifically for parasitological examination. In this study, total of 12 homologous anatomical landmarks were identified on digitised images of each specimen to get best discrimination and 26 interconnecting truss variables were generated to capture shape variation (Fig. 2). Morphometric data were collected manually and through a combination of tpsDig2 V2.1 (Rohlf, 2006b) and Paleontological Statistics (PAST) software. TpsDig2, a Windows-based program was used to digitise anatomical landmarks and to extract linear measurements from specimen images for geometric morphometric analyses.

Parasitological examination

Both ecto- and endo-parasites were examined in parasitological study. For ecto-parasites, the external surface of each fish was examined systematically with the help of a magnifying glass, paying special attention to the fin bases and folds, operculum and the buccal cavity. Gills were excised, placed in petri-dishes containing physiological saline and observed under a microscope. For endo-parasites, each fish was cut open ventrally from vent to base of operculum and the entire digestive tract was excised out. The oesophagus, stomach, and intestine were separated and slit open to release their contents into petri-dishes holding physiological saline for microscopic examination. The liver, gall bladder and intestine were also examined directly under a microscope, by preparing smears on glass slides. Whenever necessary, samples were storedat -20°C and examined later.

Statistical analysis

Before size correction, the correlation coefficients between truss measurements and standard length were close to 1. After allometric transformation for size correction (performed in MS-excel, 2016), none of the standardised truss measurements showed a significant correlation with the standard length of fish, indicating that the effect of body length was effectively removed All the morphometric measurements were log-transformed and the same data were tested for normality by using MS excel 2016 and SPSS software (SPSS 19). Size-dependent variables were removed using an allometric approach (Elliott *et al*., 1995). Data were transformed using the following formula:

Madj = M (SLmean / SL) b

where, Madj = Transformed morphometric measurement, M = Original morphometric measurement, SL = Standard length of fish, SLmean = Combined mean standard length for species, B = Within group slope of the mean regression of log M against log SL.

The transformed morphometric variables were subjected to factor analysis (FA) following the maximum likelihood method to extract the factor components. Components after varimax rotation and cumulative proportion having eigenvalues more than 0.7 were retained for further analysis. Retained factors were analysed using step-wise discriminant function analysis (SDFA) to know about how distinctly the stocks are separated. Cross-validation was performed to assess the accuracy of stock classification. Multivariate analysis of variance (MANOVA) was applied to the transformed truss morphometric variables to test for significant differences among populations using an F-test at the 0.05 level of significance. Mahalanobis distances were calculated to determine the maximum and minimum morphological separation between the stocks.

Results and discussion

Differentiation of population between coasts

Factor analysis revealed that the first two components together explained 84.64 % of total variation, with eigen values of 15.55 and 6.45 respectively (Table 1). Out of the 26 truss variables analysed, 22 variables showed significant factor loading above 0.7. Fourteen variables (13, 1-12, 2-3, 2-12, 3-4, 4-11, 4-12, 5-6, 5-9, 6-8, 6-10, 7-8, 9-10, 10-11) loaded on Factor-1, which represents overall body shape along the entire body (Fig. 3), while eight variables (1-2, 4-5,4-10, 5-10, 5-11, 6-9, 7-9, 8-9) loaded on Factor-2, reflecting body depth at the head, mid-body and caudal regions (Fig. 4).

Jayasankar *et al.* (2004) reported that, body depth and caudal peduncle depth showed high component loadings on Indian mackerel from the south Indian coast. Similarly, PCA and factor analysis effectively separated mudskipper (*B. boddarti*) stocks along the Malaysian coast (Daud *et al*., 2005). A truss network-based factor analysis of of *Decapterus russelli*, from the east and west coasts of India by Sen *et al.* (2011) revealed two morphologically distinct stocks, likely reflecting the contrasting physical and

Table 1. Eigen values and proportion of variance contribution of truss distance of *B. dussumieri*

Component	Eigen value	Cumulative values	% Total variance	Cumulative- %
1	15.55411	15.55411	59.82352	59.82352
2	6.45407	22.00819	24.82335	84.64687

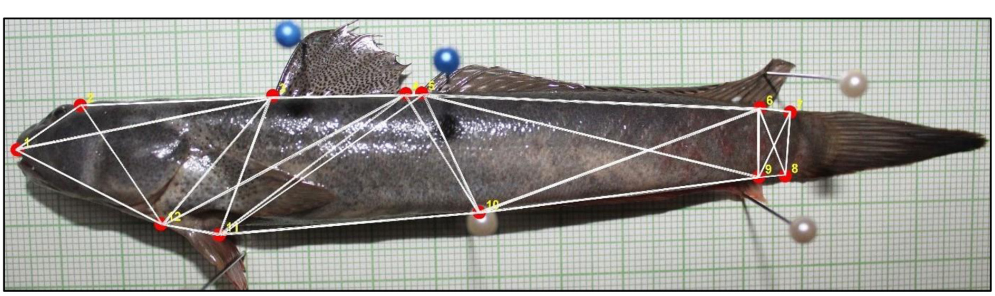

Fig. 2. Truss matrix of different land marks of *B. dussumieri*

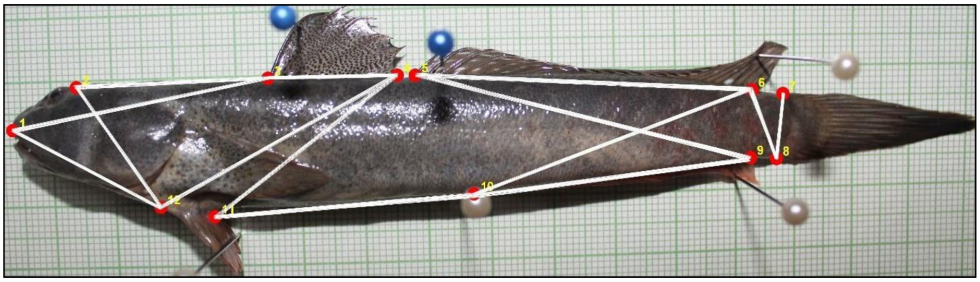

Fig. 3. Distance with meaningful loading (Factor 1) of *B. dussumieri*

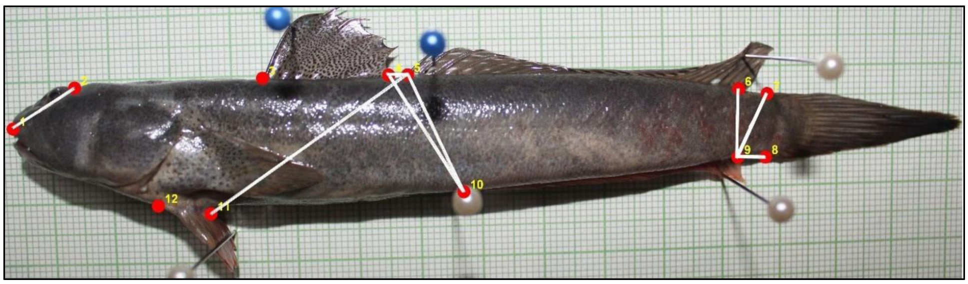

Fig. 4. Distance with meaningful loading (Factor 2) of *B. dussumieri*

ecological conditions of the Bay of Bengal and the Arabian Sea. The findings of the present study are consistent with those of Sen *et al*. (2011), as the observed separation among stocks was primarily associated with size differences. In morphometric studies, the size factor can account for 80% or more of the total in measured variables (Junquera and Perez-Gandaras, 1993). Fishes are highly responsive to environmental stimuli and can rapidly adjust their morphology in response to changing conditions.

Discrimination of observed shape differences

A subset of truss distances corresponding to Factor-1 and Factor-2 showed significant differences ($p<0.005$) in MANOVA (Table 2) and hence subjected to linear discriminant analysis (LDA) to assess the stock differentiation among the three states. All the samples collected from three locations were classified in the cross-validation of discriminate analysis. Cross-validation of LDA correctly classified 100% of all individuals from Gujarat, Maharashtra and West Bengal

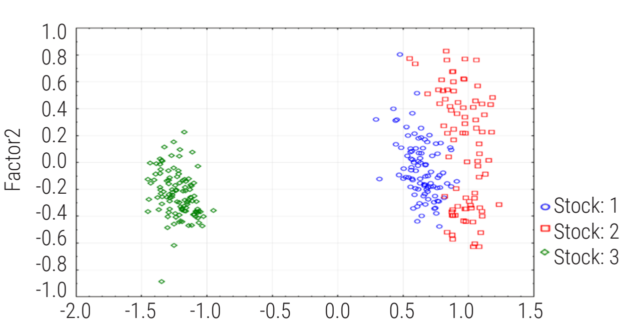

Fig. 5. Location-wise canonical discriminant group plot of two functions extracted from truss morphometric measurement of *B. dussumieri*

(Fig. 5). and both for Maharashtra and West Bengal. Mahalanobis distances confirmed significant differences among the stocks, with the greatest separation between Maharashtra and West Bengal, and the lowest between Gujarat and Maharashtra (Table 3).

Discriminant analysis of truss variables confirmed that each sampling state harbours a distinct stock of *B. boddarti*, with no evidence of mixing among regions, The findings aligns with Murdy (1989) who suggested that, high geographical isolation contributed to stock separation, as mudskippers are restricted to mangrove areas and are not continuously distributed along the coast. In mudskippers, physicochemical factors are key drivers of population differentiation (Ghanbarifardi *et al*., 2014). On the west coast, Gujarat and Maharashtra stocks showed the lowest Mahalanobis distance, while the greatest separation was noticed between west and east coast populations. This pattern may reflect differences in continental shelf structure, the inflow of organic matter, food availability and local environmental conditions. Traditional multivariate tests which rely on the Mahalanobis distances, have produced similar insights in other population studies (Faith *et al*., 1987). Comparable results were reported for Chirruh snow trout in Jammu and Kashmir, where phenotypic heterogeneity and geographic isolation limited intermixing among populations (Mir *et al*., 2013).

Stock differentiation based on parasite

Among different organs observed, only gall bladder was found infected with parasite (Table 4), which were identified as *Ellipsomyxa* sp. based on classical taxonomy (Fig. 6). Infection prevalence was lowest in samples collected from Junput and Kakdwip (West Bengal) and highest in Gujarat. Among the hosts examined, 32 were from Gujarat; 31 from Maharashtra and 17 from West Bengal, with infected fishes numbering 21, 20 and 0 respectively (Table 5).

Table 2. Multivariate analysis of variance (MANOVA) of corrected truss morphometric variable in all sampling states of *B. dussumieri*

Multivariate test of significance						
Effect	Test	Value	F	Effect- df	Error df	Sig. (p)
Intercept	Wilks	0.0	1983035	26	264	0.00
	Pillai's	1.0	1983077	26	264	0.00
	Hotellng	195298.9	1983035	26	264	0.00
	Roy's	195298.9	1983035	26	264	0.00
Stock	Wilks	0.0	2395	52	528	0.00
	Pillai's	1.9	293	52	530	0.00
	Hotellng	3778.0	19108	52	526	0.00
	Roy's	3764.1	38365	26	265	0.00

Table 3. Squared Mahalanobis distance between different stocks

Stock	Gujarat	Maharashtra	West Bengal
Gujarat	0.00	452.11	12283.67
Maharashtra	452.11	0.00	16899.41
West Bengal	12283.67	16899.41	0.00

Table 4. Qualitative occurrence of parasite group in different stations and organs of *B. dussumieri*

Stations-wise occurrence	Navasari (+)	Bhagandar (+)	Karanja (+)	Junput (-)	Kakdwip (-)
Organ or body area of occurrence	Slime (-)	Gill (-)	Gall bladder (+)	Liver (-)	Intestine (-)

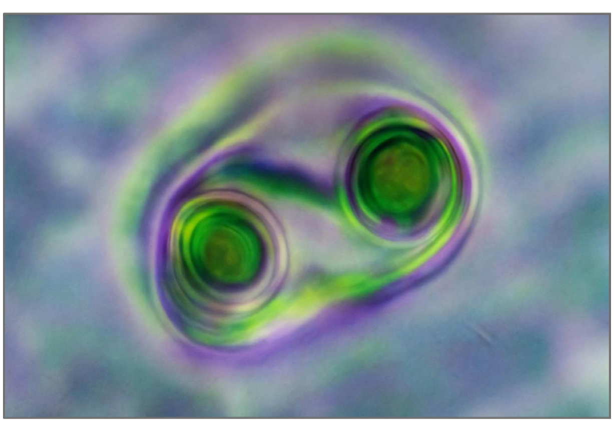

Fig. 6. Microscopic view of *Ellipsomyxa* sp. found in *B. dussumieri*

The prevalence index was relatively higher in Gujarat population (65.62%) compared to Maharashtra (64.51%). ANOVA revealed a significance difference in mean length between east and west coast populations with an F-value of 52.9, exceeding the F-critical value of 3.118 between the at 0.05 level of significance.

Among all sampling stations, only the west coast populations were found to be infected with a myxozoan parasite, identified as *Ellipsomyxa* sp. (Vandana *et al*., 2021) and infection was restricted to the gall bladder. The absence of myxosporea in east coast populations may be related to the smaller size classes present there, as parasites' occurrence often depends on host life cycle. Lester and Mackenzie (2009) opined that differences in infection levels do not necessarily indicates distinct stocks, since parasites may be seasonal or persist in a host only for a limited period. Although infection prevalence was similar between the Gujarat and Maharashtra populations and absent on the east coast, suggesting some potential for use as a biological tag, several constraints limit its reliability. Studies by Margolis *et al*. (1982) and Bush *et al*. (1997) pointed out that infection data used for stock discrimination should show significant differences among host populations and quantifiable through descriptors such as prevalence, mean intensity, and abundance. MacKenzie and Abaunza (2005) recommended that Infection indices differ clearly among different groups to qualify as effective tags, while Sindermann (1983) stressed the need for stable prevalence across seasons. In the present study, accurate counting of *Ellipsomyxa* spores was difficult, preventing estimation of parameters such as mean intensity, mean abundance and index of infection. Williams *et al*. (1992) also cautioned against using very small parasites which does not cause any visible lesions on the body or are easily confused with other species or similar particles, as their identification can be time consuming and uncertain. Moreover, the lack of distinctive morphological features complicates species-level identification and often requires molecular confirmation (Santos *et al*., 2009). Although Hemmingsen *et al*. (1991) demonstrated that myxosporeans (*e.g., Mixidium* sp. from the gall bladder) can serve as promising biological tags due to their life span exceeding 1 year in fish host. The characteristics of the *Ellipsomyxa* sp. observed in this study indicates that parasite it is not a suitable as a reliable tag for mudskipper stock identification, despite its long lifespan.

Table 5. Number of fish examined and their prevalence in different states

States	Number: examined	Number: infected	Mean length±SD	Prevalence (%)
Gujarat	32	21	14.55±0.33	65.62
Maharashtra	31	20	14.24±0.39	64.51
West Bengal	17	0	9.05± 0.25	0

Against this background, it is essential to confirm the complete absence of the parasite in fish from the east coast before considering it as a potential candidate to be used as a biological tag to distinguish east and west coast stocks. For this, further detailed long-term survey is required covering all the seasons and age groups to ensure reliable validation.

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