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Review Article

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APPLYING SINGLE VERSUS MULTIPLE METHODS FOR DELINEATION OF FISH STOCKS WITH SPECIAL REFERENCE TO MORPHOLOGICAL TOOLS

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Abstract: Stock identification methods have been developed with the progression of technology but this sometimes brings problems rather than the solution since the advancement in methods is not unidirectional but diffused. As of now, every method has pros and cons. The choice of the best method of population/stock discrimination is a critical issue depends on the frame of the study and the desired objectives to be fulfilled. The best method(s) would be the one which is rapid, reliable, low cost and easily applicable. In this article, the focus is given to morphological approaches. Since long, body morphology, as an efficient tool, has been used to delineate fish stocks, which contributes to the conservation management of important fauna. In stock or population delineation studies of fishes, landmark-based quantitative shape analysis of the organism body have a valuable role to play and complementing other existing approaches. Nevertheless, there is no single best method which can be applied to all or any species for their identification/delineation. The best method varies from species to species as does the requirement to one or more methods and even employing more than one discipline.

Keywords: Fish, Geometric morphometrics, Morphological techniques, Quantitative assessment.

INTRODUCTION

Stock or population words have been used interchangeably, although both are used in different contexts. Population structure is considered a prerequisite of conservation biology, on the other hand, stock identification is a basic element for any fishery-related study (Thorpe *et al.*, 1995; Cadrin *et al.*, 2005). In contrast to a fish population, a stock is described by management concerns defined boundaries or/and harvesting location. Interestingly, a fish stock may encompass more than one population. Stock identification is an integrative discipline, entails the identification of self-sustaining components within naturally occurring populations (Cadrin *et al.*, 2005). With spatial or temporal coherence, stocks are the distinct assemblage of fishes, having similar life-history traits and can self-reproduce with other members of a similar group (Ihssen *et al.,* 1981; Hilborn and Walters, 1992). New analytical techniques are modernizing becoming more competent to study the fish population structure (Taylor *et al.,* 2011). Fish exhibit more variations within and between populations as compared with other vertebrates, attributing to a predisposition to environmentally stimulated phenotypic divergences (Allendorf and Ryman, 1987; Wimberger, 1992). A large proportion of world fisheries occur on diverse stocks, it is essential to constantly develop new technologies to compute the various stock components that encompass these fisheries (Cadrin *et al*., 2005; Begg and Waldman, 1999). Stock identification is a multidisciplinary field and requires many techniques (Cadrin *et al*., 2005; Begg and Waldman, 1999; Waldman *et al*., 1997). It progresses along with fisheries management and conservation requirements (Begg *et al*,, 1999). Assessments of morphological characters have been one of the traditional methods of characterizing biological stocks. Morphological methods have been extensively used in fisheries research for fish phenotypic stock assessment (Hubbs and Lagler, 1947). Moreover, in the new era of phenotypic characters based management, developments in statistical tools such as truss network and geometric morphometric technology could expand the understanding from the stock to the ecosystem and may help in developing better insight about ecosystem structure. Geometric morphometrics used for shape variation has many underpinned advantages as in the understanding of behavioural differences, phenotypic, ecological, and evolutionary line. Therefore, the fisheries' evolutionary ecology and stock identification are having similar or complementary objectives (O'Reilly and Horn, 2004; Klingenberg *et al.,* 2003). Although the existence of separate reproductive populations cannot be confirmed through phenotypic methods but they can be more suitable tools for defining phenotypic stocks than genetic methods. Considering the small sum of swap over between populations that are essential to upholding genetic homogeneity might be insignificant by fishery-management context (Edmonds *et al.,*1991). Different techniques have been employed to assess variation among fish populations (Table 1, Figure 1). This article summarises some of the important techniques used in phenotypic stock delineation, with emphasis on the most commonly used approaches. In this article, the focus has been given on the three most important methods of phenotypic stock identification.

Table 1. Different techniques used to assess variations among fish populations.

Attributes	Approach	Techniques
Natural marks	Phenotypic Marker	morphological and meristic analyses
		anatomical structures
		calcified structures
		texture and spacing patterns of cerculi on otolith, scales, vertebrae
		otoliths shape analysis
		otoliths thermal marking
	Genetic analyses	genetic approaches mitochondrial DNA analysis (mtDNA)
		allozyme electrophoresis
		chromosome morphology
		microsatellites or single nucleotide polymorphism

		fatty acid profiles
	Elemental composition	otolith chemistry
		scale chemistry
	Patterns of commercial fishing	fishing seasonality
		regional catch compositions
	tagging	biological tags parasites
		electronic tags
Life history traits	Growth, timing and seasonality of reproduction	size at maturity
		timing of spawning
		distribution of larvae and eggs
		larval growth rates
		adult and juvenile growth patterns
		abundance
		maturity
		sex ratio
		reproductive health
		fecundity estimates
		gonadal development
		spawning seasons

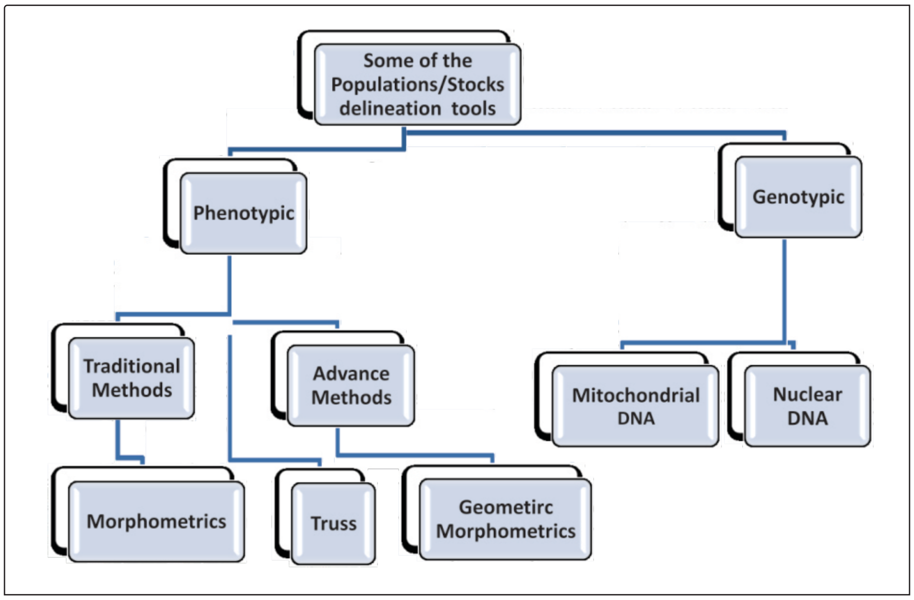

Fig 1: Tools and techniques used for delineation of fish populations/stocks.

Application of stock identification:

1. In fisheries management or restoration of species.
2. For the desirable conservation measures of threatened and endangered species.
3. To know the current state of species and to achieve sustainable yield.
4. To avoid recruitment failures and rebuild overfished stocks.
5. In the formulation of fishery management advice desirable conservation measures.
6. To estimate the stock composition in mixed-stock fisheries.

Commonly used morphological methods for fish stock delineation:

Morphological traits have long been used to delineate stocks. These traits including shape or/and meristic counts reflect phenotypic dissimilarity (Swain and Foote, 1999; Cadrin, 2005). Therefore, meristic serve as another useful tool for stock identification (Swain and Foote, 1999; Cadrin, 2005). These morphological methodologies can be applied for different body structure and shapes. These methods of fish stocks delineation/identification are economical, fast, repeatable, reliable and consistent (Pérez-Quiñonez *et al*., 2018).

SELECTION OF MORE APPROPRIATE METHOD

Several morphometric techniques are available for the quantitative assessment of morphological variation. Preferences regarding methodology to be used are often made randomly. Further, difficulty in making the choice for analysis, that which body part or whole fish body, supposed to be taken. Reportedly, the selection of features (body part) for a fish stock analysis requires correct statistical analysis tool, more than the decision of what to measure, because the selected methodology influences reliability. In the recent past many studies utilized morphological tools for stock delineation are given in the table 2.

Table 2: Summary studies of stock delineation using phenotypic characteristics.

S. No.	Fish species	Area	Populations undertaken for study	Technique	Approach	Reference
1.	*Cirrhinus mrigala*	Rivers Tons, Son, Chambal, Kalisindh, Ken, Betwa, Ganga, Sharda, Ghaghra, Gomti of India	ten	landmark based truss network morphometrics	single technique	Dwivedi *et al*., 2019
2.	*Catla catla*	Betwa, Ken, Ganga of India	three	landmark based truss network morphometrics	single technique	Sarkar *et al*., 2014
3.	*Sardinella lemuru*	Zamboanga City Philippines	two	landmark-based geometric morphometric	single approach	Echem, 2016
4.	*Decapterus russelli*	Sea, India	two	truss network analysis	single approach	Sen *et al*., 2011
5.	*Alburnus chalcoides*	Caspian Sea	four	truss network	single approach	Mohaddasi *et al*., 2013
6.	*Puntius sarana*	Rivers, the Padma, Meghna, Jamuna and the Halda in Bangladesh	four	morphometric characters	single approach	Siddik *et al*., 2016
7.	*Caspiomyzon wagneri*	two rivers southern Caspian Sea	two	morphometric characters	single approach	Vatandoust *et al*., 2015
8.	*Oreochromis* spp.	Philippine fisheries institutions	four	truss measurements	single approach	Regala *et al*., 2018
9.	*Rutilusru tiluscaspicus*	Sin southern Caspian Sea Bandar-e-Turkmen shore, Anzali wetland and Aras River	three	geometric morphometric	single approach	Ghojoghi *et al*., 2014

10.	*Amblygaster clupeoides*	Bay of Bengal coast, in Bangladesh	four	truss network technique	single approach	Hanif *et al.*, 2019
11.	*Channa punctatus*	Gomti River, ponds situated at Kolkata, Malihabad	three	truss analysis	single approach	Kashyap *et al.*, 2016
12.	*Sperata aor*	Ganga River, viz. Narora, Kanpur, Varanasi and Bhagalpur	four	truss network	single approach	Khan and Nazir, 2019
13.	*Chalcalburnus chalcoides*	Estuaries Haraz River, Shirud River	two	truss network	single approach	Bagherian and Rahmani, 2009
14.	*Puntioplites bulu*	Peninsular Malaysia Kelantan River, Perak River and Pahang River	three	truss network analysis	single approach	Ghani *et al.*, 2018
15.	*Xenentodon cancila*	Boluhorpurbao, Jhenaidah, Bhairab River, Jashore, Arial Khan River, Madaripur, and Bohnnibaor, Gopalganj in Bangladesh	four	truss network system	single approach	Sarower-E-Mahfuj *et al.*, 2019
16.	*Ponticola bathybius*	Iranian waters of the Caspian Sea	three	landmark-based geometric morphometric and meristic analysis	multiple approach	Tajbakhsh *et al.*, 2018
17.	*Mullus surmuletus*	Eastern English Channel, Bay of Biscay	two	truss analysis and length measurements	multiple approach	Mahe *et al.*, 2014
18.	*Clarias batrachus*	Ganga (Narora and kanpur) and its tributaries: Yamuna and Gomti rivers	four	truss morphometric of fish body and variation in otolith chemistry	multiple approach and interdisplinary	Miyan *et al.*, 2016
19.	*Cirrhinus reba*	Brahmaputra, the Padma, the Karatoya, and the Jamuna Rivers in Bangladesh	four	meristic characters morphometric characters truss measurement	multiple approach	Ethin *et al.*, 2019
20.	*Opisthonema libertate*	Magdalena Bay, Guaymas, and Mazatlan, Mexico	three	geometric morphometrics based on body and otolith shape	multiple approach	Pérez-Quiñonez *et al.*, 2018
21.	*Dentex dentex*	Corsica Island, four zones: Cap Corse, Galeria, Ajaccio, Bonifacio	four	microsatellite DNA markers, otolith shape analysis and parasites communities	multiple approach and interdisplinary	Marengo *et al.*, 2017

MORPHOLOGICAL ANALYSIS TOOLS

1. Traditional morphological analysis

Morphology incorporates both morphometric and meristic study that is the most commonly used taxonomic tools for the separation of species and population. Several workers have used these techniques for taxonomic identification of fishes (Ihssen *et al.,* 1981; Melvin *et al.,* 1992; Quilang *et al.,* 2007). Morphometric and meristic morphological characters are the simple, most direct and frequently employed methods to delineate stocks of fish (Mamuris *et al.,* 1998; Bronte *et al.,* 1999; Hockaday *et al.,* 2000). Traditional methods of fish stock identification have served up immensely in fisheries management since ages and now also carrying out the same, nonetheless with the advancement in technology additional/substitute methods including other scientific disciplines with different traits coming into existence (Brander, 2003). These traditional methods having limitations too, like through these methods only linear distances around the body can be measured as this focuses on the measurements along the particular axis of the body (fish) thus in one direction hence, non-uniform coverage of the fish body. Some of the morphological measurements are standard length, body depth at the dorsal-fin origin, mandibular length, upper jaw length, body depth at the dorsal-fin origin, head length, pre-dorsal length, pelvic fin length, pre-anal length, pre-pelvic length, pre-maxillary teeth and head width, dorsal-fin base, anal fin base, peduncle length, peduncle depth, snout length. These techniques consist of principal component analysis, principal coordinate analysis, factor analysis, discriminant analysis, canonical variate analysis, and multivariate analysis of variance (Rohlf and Marcus, 1993; Adams *et al.,* 2004).

2. Truss based morphological analysis

To overcome the limitation of traditional techniques, new technological advancement has been made that is facilitated by image processing methods, more inclusive and accurate data collection, more efficient quantification of shape, and new analytical tools (Cadrin, 2000). Truss network system (Strauss and Bookstein, 1982; Bookstein *et al.,* 1985) is a landmark-based technique, measurements generated are a chain of distances estimated linking landmarks that construct a pattern of connected quadrilaterals across the body structure (Strauss and Bookstein, 1982). The truss morphology analysis is a good tool for observing information on the appearance of an organism (Cavalcanti *et al.,* 1999). A digital picture is useful in the long term as it can obtain morphometric data and the potential for reprocessing each individual to confirm unusual measurements or accomplish substitute/ additional sets of characteristics. Storage of picture also allows detailed examination of extreme variants or outliers, as well as more flexible characteristic selection (Cadrin and Friedland, 1999).

3. Geometric morphometric analysis

Geometric morphometrics has turned into the leading techniques to quantify differentiation in the shape of biological bodies (Klingenberg, 2010). The innovative introduction of geometric morphometrics in stock analysis has additionally been able to overcome multiple barriers imposed by subjectivity. This presents a powerful tool for the construction of fish stock delineation models, presenting a series of efficient tools for the processing of complex data. It involves the analysis of configurations of discrete anatomical loci (landmarks) among individuals and has been applied to several questions. In general, landmark positioning is first executed by hand on individual images. Procrustes superimposition and various multivariate statistics can be applied to distinguish variations in landmark pattern and consequently shape changes in populations (Vandaele *et al.,* 2018; Lorenz *et al.,* 2017).

PHENOTYPIC VARIATION AND STOCK IDENTIFICATION

Despite current progress in molecular techniques, morphological assessment is remaining the leading approach and the first important step for examining the stock structure of fishes (Costa *et al.,* 2003; Solomon *et al.,* 2015). Phenotypic markers may recognize morphological discrimination which is induced by environmental differences in the moderately isolated stocks, practical level of portioning among self stock recruitments. Such self-recruiting stocks independently react to the exploitation of species even without showing any genetic differentiation (Carvalho and Hausar, 1994). The main advantage of using morphological characters in studies of stock/ population structure is that these characters are often related to fitness and respond to selection, and thus may disclose genetic differentiation not obvious in neutral genetic character. Phenotypic traits are typically most useful when multiple traits are investigated to study short-term, environmentally induced variation (Begg *et al.,* 1999). Phenotypic plasticity is one of the disadvantages of using morphological characters for population studies (Debat and David, 2001). Phenotypic variation is owing to both environmental and/or through inheritance. Distinguishing between them is the basic difficulty that should be addressed when using these characters to examine population/stock structure (Swain and Foote, 1999; Begg *et al.,* 1999).

SINGLE TECHNICAL APPROACH VERSUS A HOLISTIC APPROACH

The argument in favour of single approach

In stock delineation, "more is not essentially better" a single technique that gives accurate and precise result may give better result than applying many techniques using different parameters and coming up with the discordant result. Multiple approaches exercised in stock delineation may show different patterns of the stock structure and combining the outcome can be complicated which lead to more confusion in declaring the unanimous result. Therefore, using multiple methods for one questionable stock identification problem may give conflicting outcome about the stock structure (Cadrin *et al.,* 2013; Izzo *et al.,* 2016).

The argument in favour of multiple approach

A wide study for stock identification should incorporate several techniques that accounts to diverse aspects of the stock concept appropriate to scientists, fish farmers and managers. No single stock definition can incorporate all dynamic such as environmental, biological, political and the useful definition has to adjust with the management endeavour (Coyle, 1998). Therefore, single technical approaches are inadequate to delineate complex fish stocks. There is a necessity to exploit the potentially of complementary approaches and tools because employing an approach may underused (Pita *et al.,* 2016). Applying two or more methods of the same discipline is probably give a better and unanimous result. Combination of multiple approaches together could give complementary insights and a prospect to compare the utility of each of them and the potential to understand population interaction in a different context (Marengo *et al.,* 2017).

CONCLUSION

Although multiple methods can provide conflict outcomes, such conflict cannot be dismissed by selecting single approach. It is not easy to select the best method, the best approach varies from species to species as does the need to use one or more methods or even more than one discipline.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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