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REVIEWS

Advancements in morphometric differentiation: a review on stock identification among fish populations

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Abstract Identifying intraspecific units or stocks of a species with unique morphological characteristics has now become more powerful and enables a better management of these subunits of species and ensures better management and conservation of the fishery resources. These morphometric characteristics typically show ontogenic changes in body shape particularly rapid at key life history stages. For about the past 50 years, traditional multivariate morphometrics, accounting for variation in size and shape, have successfully discriminated many fish stocks throughout the world, however, they have always been criticized because of several biases and weaknesses. To contribute to the advancement of fish stock identification, a new technology based on "Truss Network System" has emerged as a new tool with more effective strategies for descriptions of shape, better data collection and diversified analytical tools. In the present communication, recent developments made in the discipline of morphometric differentiation in body shape among fish populations are briefly reviewed and it appears that the truss based techniques has now been proved to be more effective than manual distance measurement for the management of fishery resources throughout the world. However in India, these techniques have not been commonly applied in fisheries research to discriminate fish stocks. The study expands the potential through various advancements in morphometric differentiation analysis which could serve as a tool for stock identification among fish populations.

Keywords Stock identification · Morphometry · Fish population

Introduction

Stock identification is an interdisciplinary field that involves the recognition of self-sustaining components within natural populations (Cadrin et al. 2005). A fundamental requirement of this approach is to consider the full impact of management actions, including identifying the stock complexity of a fish species (Begg et al. 1999). Because stock structure and delineation are uncertain, the reliability of stock assessments, and therefore the effectiveness of fishery management, is severely limited for many fishery resources (Cadrin et al. 2005). Therefore, to manage a fishery resource rationally and effectively, it is essential and important to know the stock structure of an exploited species, as each stock must be managed separately to optimize their yield (Grimes et al. 1987).

Several techniques have been proposed for stock identification that involves meristics, morphometrics,

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traditional tags, parasites as natural tags, otolith chemistry and several molecular markers (e.g., protein allozymes, mitochondrial DNA, microsatellite DNA), however, morophometric traits proves to be the most frequently employed and cost-effective methods (Sajina et al. 2011). Stock identification methods have developed in parallel with the advancement of morphometric techniques. Morphometric variability among different geographical populations are attributed either to distinct genetic structure or to environmental conditions in each area (Kinsey et al. 1994). Organisms, therefore, having the same morphometric characteristics, are often assumed to constitute a stock.

Morphometric variation between stocks can provide a basis for stock structure, and may be more applicable for studying short-term, environmentally induced variation for better fisheries management (Begg et al. 1999). However, morphological features of an organism are not autonomous and changes in various aspects of morphology are coordinated (Zelditch et al. 1992). Consequently, unless a specific morphometric characteristic is known to have a genetic foundation, morphology is best described by multivariate techniques (Thrope and Leamy 1983). Using fishery data is the least satisfactory method to analyze the stock structure because the data are not collected for racial studies, and the derivative result must be confirmed by different methods (Nishida 1992). It is common for stock structure to be indicated by morphological characteristics but not by isoenzymes (Ryman et al. 1984). Indeed, it may be that enzyme electrophoresis is too weak as a tool to reveal genetic differences (Ferguson et al. 1995).

Stocks characterized on the basis of morphometric traits are quantitative genetic traits typically controlled by many genes and affected by the environment in which those genes are expressed (Falconer 1981; Hard 1995). They also are generally related to fitness and thus, molded by natural and sexual selection, they reflect local adaptation (Carvalho 1993; Hard 1995; Conover 1998). However, the importance of delineating groups of fish characterized by phenotypic differences that may be entirely environmentally induced, is being increasingly emphasized (Shepherd 1999; Haddon and Willis 1995; Jerry and Cairns 1998; Lowe et al. 1998; Cadrin 2000). Cadrin and Friedland (1999) argue that intraspecific groups with persistent phenotypic differences in life history traits need to be recognized in stock assessment and fisheries management, even if these differences do not reflect genetic differentiation.

Traditional multivariate morphometrics, accounting for variation in size and shape, have successfully discriminated many fish stocks (Turan 1999). However, traditional methods have been enhanced by image processing techniques, through better data collection, more effective descriptions of shape, and new analytical tools. The development of image analysis systems has facilitated progress and diversification of morphometric methods and expands the potential for using morphometry as a tool for stock identification (Cadrin and Friedland 1999). Truss network is much more powerful in identifying intraspecific groups with different life history stages according to shape variation than manual measurements (Corti et al. 1988; Strauss and Bookstein 1982; Bookstein 1982).

The use of morphometric landmark characteristics to identify phenotypic stocks, with the advancement of imaging technology and analytical techniques have increased the power of morphometric analysis for stock discrimination and stock composition analysis. This review article is intended to provide the recent developments made in the discipline of morphometric differentiation among fish populations; therefore specific details are provided concerning the morphometric landmarks, statistical analysis, and influential factors involved in stock identification.

Morphometric landmarks

The result of a morphometric analysis depends on the particular set of measurements chosen. Morphometric distances can be measured or calculated between "morphometric landmarks". Morphometric landmarks are homologous, representing the same developmental features among specimens and could be easily located. If the selection of distance measures does not correspond to the principal directions of shape differences, the resulting descriptions of the differences between forms will be inadequate (Strauss and Bookstein 1982).

Traditional measurements

For the past 50 years, morphometric investigations have been based on a set of traditional measurements described by Hubbs and Lagler (1947). Morphometric

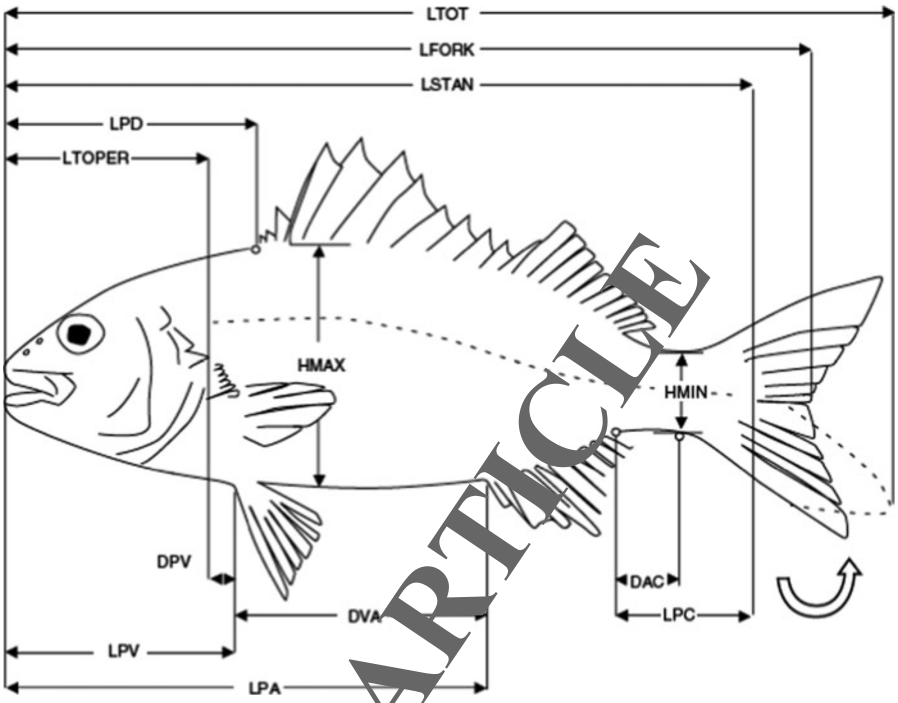

Fig. 1 Morphometric measurements of silver perch, *L. plumbeus* (*Source*: Quilang et al. 2007)

measurements (Fig. 1) are taken on the left side of the body using the conventional orthogonal methods between the perpendicular and horizontal plane, not following the curves of the body (Surre et al. 1986). Descriptions of the 35 morphometric characteristics are given in Table 1.

These measurements have recently been criticized because they are concentrated along the body axis with only sampling from depth and breadth, and most measurements are in the head (Turan 1999). Furthermore, individual measurements often extend over much of the body, and some morphological landmarks such as the tip of snout and the posterior end of the vertebral column are used repeatedly as a central point for most of the measurements. Thus these traditional measurements represents a biased coverage of body form (Strauss and Bookstein 1982), and success in selecting effective characteristics has been attributed to a matter of chance (Bookstein 1982).

Truss network measurements

The 'Truss networks' of distances between landmarks coordinates covers the entire fish in a uniform network, and theoretically increase the likelihood of extracting morphometric differences with greater discriminating power. To overcome the inherent weakness of traditional morphometric methods, a system of morphometric measurements called the "Truss Network System" (Strauss and Bookstein 1982) with properly calibrated coordinates of morphometric locations, or 'landmarks' has been increasingly employed for the purpose of stock identification, which essentially discriminates 'phenotypic stocks' that are groups of individuals with similar growth, mortality and reproductive rates (Booke 1981). The methodology is predicated on the measurement of across-body distances connecting two morphological landmarks from a sequential series of connected polygons. This type of landmark-based technique using geometric morphometrics imposes no restrictions on the direction of variation and localisation of shape changes and is highly effective in capturing information about the shape of an organism (Cavalcanti et al. 1999).

Digital images archived by calibrated digital cameras provide superior data format, accuracy, design flexibility, and potential for substantially increasing sample size. Coordinates of digital images can be calibrated to unit distances if specimens are placed on a plane with a grid of known distances (truss sheet or graph paper) for a standard view (usually lateral or

Table 1 Definitions of morphometric measurements of silver perch *L. plumbeus*

LTOT	Maximum total length
LFORK	Fork length
LSTAN	Maximum standard length
LPA	Pre-anal distance
LPV	Preventral distance
LPD	Pre-dorsal fin distance
LTOPER	Head length, from top of mouth to edge of operculum
LPREO	Preorbital distance
LPOSO	Post orbital distance
HOR	Orbital depth of head
HOC	Occipital depth of head
DHOE	Horizontal diameter of eye
IT	Interorbital thickness measured at level of upper edge of orbits
LM	Lower jaw length
LU	Upper jaw length
LMAXIL	Maxillary width
HMAX	Maximum body depth
HMIN	Minimum body depth
BTMAX	Maximum body thickness measured on lateral line perpendicular to start of dorsal fin
LD	Base length of dorsal fin
HDANT1	Anterior height of dorsal fin corresponding to length of fourth dorsal spine
HDANT2	Anterior height of dorsal fin corresponding to length of 11th spine
HDPOS1	Posterior height of dorsal fin corresponding to length of second dorsal soft ray
HDPOS2	Posterior height of dorsal fin corresponding to length of seventh dorsal soft ray
DPV	Distance between pectoral and ventral fins
DVA	Distance between ventral and anal fins
DAC	Distance between anal and caudal fins
LPC	Caudal peduncle length, from end of base of anal fin
LCMED	Length of middle caudal rays
LCSUP	Length of upper lobe of caudal fin
LCINF	Length of lower lobe of caudal fin
HA	Height of anal fin
LA	Base length of anal fin
LV	Length of ventral fin
LP	Length of pectoral fin

Source: Quilang et al. 2007

dorsal) (Fig. 2). One major advantage of deriving morphometric data from digital images is the ability to store the image and the potential for reprocessing each individual to confirm anomalous measurements or derive alternative sets of characteristics. Storage of images also allows detailed inspection of extreme variants or outliers, as well as more flexible characteristic selection (Cadrin and Friedland 1999).

Truss networks are constructed by interconnecting the landmarks (Fig. 3). Morphometric distances can be calculated from digital landmarks coordinates covering entire shape of the fish. Landmarks should be homologous, representing the same developmental feature among specimens, and should be easily located (Winans 1987; Bookstein 1990). The extraction of the truss distances from the digital images of specimens can be generated using a linear combination of three software platforms, tpsUtil, tpsDig2 v2.1 (Rohlf 2006) and PAleontological STatistics (Hammer et al. 2001).

Fig. 2 Locations of the *12* landmarks for constructing the truss network on fish illustrated as *open circles* and morphometric distance measures between *circles* as *lines*. Landmarks refer to *1* anterior tip of snout at upper jaw, *2* most posterior aspect of neurocranium (beginning of scaled nape), *3* origin of dorsal fin, *4* insertion of dorsal fin, *5* anterior attachment of dorsal membrane from caudal fin, *6* posterior end of vertebrae column, *7* anterior attachment of ventral membrane from caudal fin, *8* insertion of anal fin, *9* origin of anal fin, *10* insertion of pelvic fin, *11* insertion of pectoral fin, *12* posterior most point of maxillary

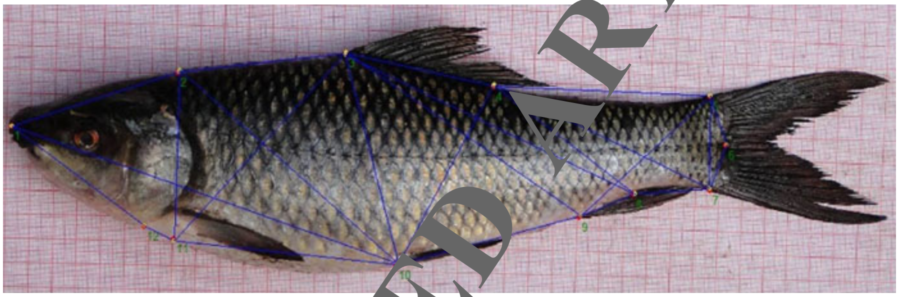

Fig. 3 Truss network on fish

Statistical analysis

Morphometric characteristics with continuous variables with meaningful correlations are therefore appropriate for conventional multivariate analysis (Blackith and Reyment 1971; Reyment et al. 1984; Marcus 1990; Klingenberg 1996). The earliest analyses of morphometric variables for stock identification were univariate comparisons, but these were soon followed by bivariate analyses of relative growth to detect ontogenetic changes and geographic variation among fish stocks. As the field of multivariate morphometrics grew, a set of multivariate methods was applied to quantify variation in growth and form among stocks (Cadrin 2000). Studies on multivariate allometric patterns may be traced to the pioneering work of Jolicoeur (1963). In a multivariate sense, morphology has two independent components: size and shape (Bookstein et al. 1985). Allometric analysis is used to assess geographically induced variation in size and provides a method to elucidate the relationship between processes of growth and evolution (Blackstone 1987; Klingenberg 1996). Most of the variability in a set of multivariate characteristics is due to size (Junquera and Perez-Gandaras 1993). Thus, shape analysis should be free from the effect of size to avoid misinterpretation of the results (Strauss 1985). Therefore, transformation of absolute measurements to size–independent shape variables is an important stage in the data preparation for morphometric analysis (Reist 1985). However, there is no need to eliminate the size effect if the samples for all populations are from same ages (Turan 1999).

Several univariate and multivariate techniques are being used to remove the effect of size, e.g. regression analysis, allometric method and the most frequently used multiple group principal component analysis (MGPCA) (Klingenberg 1996). Base-10 logarithms are used for all variables. Standard length is used for all cases, since it correlates strongly with other morphometric characteristics (Reist 1985; Cambell 1976). The efficiency of size adjustment transformations can be assessed by testing the significance of correlations between the transformed variables and the standard length. A significant correlation indicates an incomplete removal of size effects from the data (Turan 1999).

Principal component analysis (PCA) is another valuable diagnostic and exploratory tool to eliminate the size effect, to identify statistical outliers, assess normality (or log normality), and inspect linearity (or log linearity) of correlations (Cadrin 2005). On the basis of multidimensional growth, correlation among log-transformed distances and resulting principal components can be interpreted in terms of isometric size variation and allometric shape variation (Teissier 1960; Jolicoeur 1963; Reyment 1990; Klingenberg 1996). If all characteristics are positively correlated and loaded nearly equally on the first principal component (i.e., all are similarly correlated with the PC1 score), PC1 can be interpreted as isometric size and scales the relative size of the specimens. Differences in size distribution among putative stocks may result from growth or mortality differences among areas and need to be considered in discriminations so that classification is based on shape differences rather than size differences (Cadrin 2005). For, example, a recent study that discriminated sturgeon species was found to be invalid because it incorrectly classified individuals to species based on size (Rincon 2000). Therefore, to eliminate the size effect in principal components analysis (PCA), the first principal component (PC) should be eliminated, as the first PC expresses size variation, while the others express genuine variation among stocks. Weight unit can also be used for eliminating the size effect (Corruccini 1983), though weight is a power of length (Ricker 1975). In this way, each morphometric measurement is divided by the individual gutted weight prior to multivariate analysis. Several methods of multivariate size correction have been developed, but Burnaby's (1966) method, which involves the removal of with-in group multivariate size, appears to be the most appropriate (Rohlf and Bookstein 1987; Klingenberg 1996).

Morphological differentiation may vary between the sexes in some fish species (Turan 1999). Creech (1992) reported greater variation between two sand smelt species (*Atherina boyeri* and *A. presbyter*) in females than in males while Hossain et al. (2010) observed no significant differences of tested variables between the sexes within the same stock. Therefore, the interaction between variables and sexes should also be tested. In the case of any significant correlation, females and males should be treated separately in multivariate analyses to remove the effect of sex from the result.

The transformed data can be subjected to various multivariate techniques; principal components analysis (PCA), factor analysis, cluster analysis and multiple-discriminant function analysis (DFA) or canonical analysis (CA) using a statistical package program (e.g. SPSS 2011; SYSTAT 2002; PAST 2001; SAS 1990; R 2006) and graphs can also be generated using these programs (Turan 1999; Hatcher 2003; Cadrin 2005). Multivariate techniques simultaneously consider the variation in several characteristics and thereby assess the similarities between samples (Turan 1999).

Principal component analysis (PCA)

Within-group, PCA is an effective method for detecting statistical outliers from processing errors or abnormal morphometric development (Cadrin 2005). Pooled-group PCA is a powerful exploratory tool for examining patterns of morphometric variation and choosing characteristic sets that may efficiently discriminate groups (Cadrin 2005). However, PCA requires no a priori grouping of individuals but combines and summarizes the variation associated with each of a number of measured variables into a smaller number of principal components (PCs) which are a linear combination of the variables that describe the shape variations in the pooled sample (Turan 1999). Correlations between original variables and the principal components (component loading) can be used to interpret the importance of individual variables in the description of the variation of the data set (Turan 1999). The second principal component accounts for the maximum amount of variation remaining after isometric size variation is removed by PC1, and therefore measures shape variation. Characteristics that load strongly positive or strongly negative on PC2 have large influence and reveals shape contrasts. Factor and Cluster analysis are also useful for exploring patterns of shape variation in size-adjusted data, but interpreting group differences is more difficult than PCA results.

Discriminant function analysis (DFA)

Significance of morphometric differences among putative stocks is commonly tested using multivariate analysis of variance or discriminant function analysis (Cadrin 2005). However, multivariate tests with a large number of morphometric characteristics and many observations are extremely sensitive, and statistical significance may be spurious (Misra and Easton 1999). A more, meaningful criterion for detecting differences is the ability of a discriminant function to classify extrinsic specimens to the correct stock with greater accuracy than random classification (Solow 1990). DFA requires a priori grouping of samples calculates a function discriminating between samples of known identity and then reclassifies the individuals into the designated groups on the bases of this function (Turan 1999). The percentage of correctly classified individuals gives a measure of the morphological distinctness of the samples. Pooled within-groups correlations between variables and discriminant scores can be used to interpret canonical variants, similar to the way PCA loadings are interpreted (Cadrin 2005). Plotting truss networks or thin-plate spline deformations as canonical variate score can also help to interpret discriminant functions (Sheehan et al. 2005; Cadrin and Silva 2005).

Factors underlying intraspecific patterns of morphometric characteristics

Morphometric differences within a species are generally linked with geographical isolation due to the interactive effects of environment, selection, and genetics on individual ontogenies (Poulet et al. 2005). Morphometric characteristics typically show ontogenetic changes associated with allometric growth (Gould 1966). These ontogenetic changes in body shape may be particularly rapid at key life history stages, such as metamorphosis from larval to juvenile body forms, smoltification and sexual maturation (Swain et al. 2005).

Environmental influences

Phenotypic variations in morphological characteristics are more applicable to study short-term environmentally influenced differences between fish stocks (Lindsey 1962; Ali and Lindsey 1974; Todd et al. 1981; Rohlf and Marcus 1993; Wainwright et al. 1991; Swain and Foote 1999). Although environmental influences on morphometric characteristics have not been well studied, a number of influential factors have been identified (Swain et al., 2005). Haas et al. (2010) found that the physical characteristics of habitats drive changes in the morphological attributes of native fish populations. Body shape in fishes can be modified by rearing temperature (Martin 1949; Beacham 1990), quantity of food (Currens et al. 1989) and type of food or feeding mode (Meyer 1987, 1990; Witte et al. 1990; Wimberger 1991, 1992; Wainwright et al. 1991; Day et al. 1994; Robinson and Wilson 1995; Day and McPhail 1996; Pakkasmaa 2001; Peres-Neto and Magnan 2004; Proulx and Magnan 2004). A study by Imre et al. (2002) demonstrated morphological variation in the caudal area in brook charr (*Salvelinus fontinalis*) from microhabitats differing in water velocity. They observed a deeper caudal peduncle in fishes from turbulent waters. Sajina et al. (2011) reported that the variation in the caudal region of specimens of *Megalaspis cordyla* from the Arabian Sea and the Bay of Bengal could be consequence of phenotypic plasticity in response to uncommon hydrological conditions between these areas. The more turbulent water conditions in the Bay of Bengal than the Arabian Sea (Kolla et al. 1976; Chamarthi et al. 2008) may explain the variation in the caudal region observed. Structure made of bone remodel and change shape depending on the stresses imposed on them (Lanyon 1984; Lanyon and Rubin 1985). These changes are usually considered to be adaptive (Lanyon and Rubin 1985). Plasticity in trophic morphology induced by diet or feeding mode is usually assumed to result from bone remodeling in response to differences in loading regime (Swain et al. 2005). Other environmental influences may involve heterochrony, changes in the relative timing of developmental events (Meyer 1987) such as switches between growth stanzas (Martin 1949).

Phenotypic plasticity in morphometric traits may often be adaptive (Robinson and Parsons 2002). In the presence of northern pike, *Esox lucius*, predator-induced changes in body shape of crucian carp, *Carassius carassius* provide a shrinking example of adaptive plasticity by developing a deeper body (Bronmark and Miner 1992). Adaptive plasticity may also contribute to the morphological differences between the benthic and limnetic forms observed in a variety of fish taxa (Robinson and Wilson 1994; Robinson and Parsons 2002; Sacotte and Magnan 2006; Knudsen et al. 2007; Bertrand et al. 2008). The limnetic forms have more and longer gill rakers; shallower bodies and heads; longer heads, snouts, and upper jaws; and larger eyes than the benthic forms (McPhail 1984, 1992). Common environment experiments indicate that the differences in morphology are inherited (McPhail 1984, 1992; Hatfield 1997), but the two forms also exhibit morphological plasticity in the adaptive direction (Day et al. 1994). Diet-induced plasticity resulted in improved foraging efficiency (Day and McPhail 1996) and reduced the morphological gap by 30–60 % (Day et al. 1994). Similar diet-induced plasticity has been demonstrated in the cichlids *Geophagus brasiliensis* and *G. steindachneri* (Wimberger 1991, 1992). Similarly, changes in feeding orientation induced morphological differentiation in guppies *Poecilia reticulata* (Robinson and Wilson 1995). Morphological differentiation associated with trophic specialization has also been extensively studied in Arctic char *Salvelinus alpinus* and also involves both genetic differences and phenotypic plasticity (Skulason et al. 1989, 1993, 1996; Snorrason et al. 1994). Meyer (1987) reported extensive phenotypic plasticity in trophic morphology resulting from differences in diet and feeding mode in the cichlid *Cichlasoma managuense*. Adaptive phenotypic differences between the groups of fish may thus reflect plasticity instead of indicating genetic differentiation between the groups (Swain et al. 2005).

Genetic influences

Morphometric traits are quantative genetic characteristics, generally thought to be influenced by many genes of small individual effect, though some adaptive morphometric differences may be explained by relatively few genes of large effect (Hatfield 1997). Estimates of the heritability of morphometric characteristics range between low and moderate values. Riddell et al. (1981) reported heritabilities less than 0.1 for morphometric traits in Atlantic salmon. The average heritability reported for morphometric characteristics of chum salmon ranged between 0.3 and 0.6, depending on rearing temperature (Beacham 1990). Lavin and McPhail (1987) reported heretabilities between 0.19 and 0.84 for morphometric characteristics in the threespine stickleback *G. aculeatus*. Grudzien and Turner (1984) reported a heritability of 0.44 for the mouth width polymorphism in *Ilyodon*. Genetic variation for morphological plasticity has also been demonstrated whenever it has been tested in fishes (Robinson and Parsons 2002).

Overview of morphometric variation among populations

Stock identification studies have played a central role in the development of traditional landmark methods (Cadrin 2000) which have been well documented from the earlier studies on bivariate allometry of crustaceans and finfish in the 1920s reviewed by Huxley (1932) and Teissier's (1960). Royce (1957) reviewed methods of multivariate morphometrics for studying subpopulations of fishes more than a decade before general texts on morphometrics were published (Blackith and Reyment 1971; Pimentel 1979). The proliferation of morphometric applications for stock identification during the 1970s and 1980s is illustrated by the case studies reviewed by Lee (1971), Winans (1987), and Saila and Martin (1987), as well as subsequent increase in published case studies (Fig. 4). Early investigators used descriptive statistics and univariate analyses separately on each morphometric characteristic, but these did not always yield efficient results (Surre et al. 1986). Thus, multivariate techniques such as principal component analysis, factor analysis, cluster analysis, and discriminant analysis, have been adopted in the study of population structure of fishes (Ihssen et al. 1981; Surre et al. 1986; Hedgecock et al. 1989; Melvin et al. 1992; Mamuris et al. 1998; Trapani 2003).

Sumantadinata and Taniguchi (1990) studied the morphological variation among seven common carp stocks collected from West Java, East Java and West Sumatra and found that the Majalaya and Punten stocks were significantly greater in body depth, intestine length and number of vertebrae than the other stocks. The body width of Majalaya was the highest for all the stocks. Mamuris et al. (1998) examined morphometric variation in 15 characteristics of the red mullet *Mullus barbatus* samples from seven Greek localities using multivariate analysis. ANOVA, stepwise discriminant analysis and cluster analysis revealed a rather high morphological variability between the populations studied. The discriminant analysis revealed that about 80 % of the

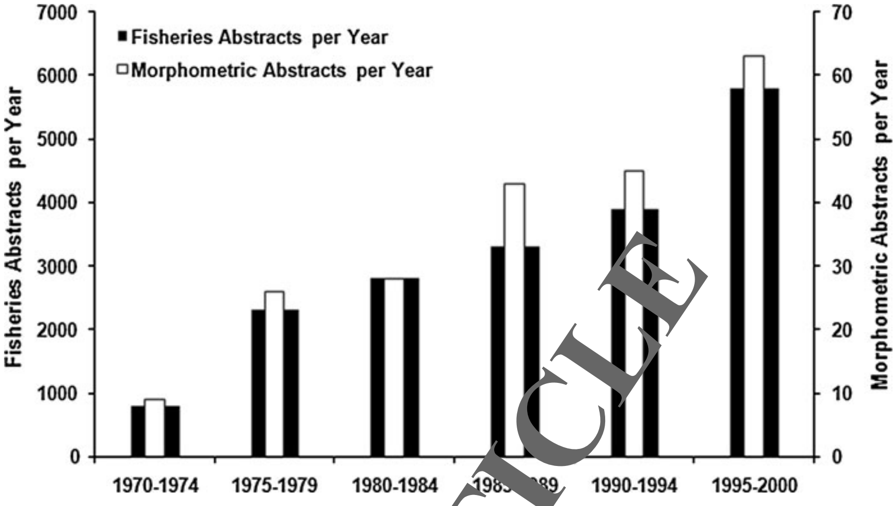

Fig. 4 Frequency of published case studies on morphometric stock identification by 5-year period, with fishery references for comparison (*data source*: Cambridge Scientific Abstracts, Aquatic Sciences and Fisheries, Biological Sciences and Living Resources, http://www.csa2.com)

examined fish could be correctly classified into the seven areas. This variability could be explained in terms of genetic structuring of the populations and/or environmental conditions prevailing in each geographic area in combination with fish migration and egg/larvae transportation from one area to another. Murta (2000) investigated the morphological variation of horse mackerel (*Trachurus trachurus*) in the Iberian and North African Atlantic using multivariate techniques such as hierarchical cluster analysis, multivariate discriminant analysis and randomization tests using some 14 morphometric and five meristic characteristics. Morphometric characteristics showed considerably greater discriminatory power to distinguish individuals from different areas than did the meristic characteristics. Meristic characteristics did not allow a clear distinction between geographical areas, with the Mahalanobis distances (a measure of divergence or distance between groups in terms of multiple characteristics) among areas being an order of magnitude smaller than those for the analysis of the morphometrics. Walsh et al. (2001) quantified morphological variation among Shortnose Sturgeon *Acipenser brevirostrum* from Kennebec and Androscoggin Rivers, Maine and Hudson River, New York using six morphometric characteristics and provided evidence for distinct populations. Turan et al. (2005) examined the pattern of morphometric differentiation among six populations of *Clarias gariepinus* sited in the Asi, Seyhan, Ceyhan, Göksu, Aksu, and Sakarya river systems in Turkey and significant differences were observed between means of the six samples for 18 out of 20 standardized morphometric measurements using univariate analysis of variance. Ibáñez-Aguirre et al. (2006) studied comparative morphometrics of two populations of *Mugil curema* on the Atlantic and Mexican Pacific coasts with nine morphometric variables using normalization of the individuals of each group and two multivariate methods viz. correspondence analysis and discriminant analysis. Results indicated that the diameter of the eye differentiated the populations of both coasts, the Atlantic population showed a larger eye diameter. Morphological variation of the euryhaline cichlid fish *Etroplus suratensis* (Bloch) from six geographically separated estuarine localities along the southern and western coasts of Sri Lanka was studied by Suneetha Gunawickrama (2007) and significant heterogeneity in morphology of the cichlid was recorded with respect to nine morphometric characteristics. Fish of Nilwala estuary and Garanduwa lagoon were not significantly different in morphology, yet they show discernible differences from the other four samples (Kahanda lagoon, Chilaw lagoon, Walawe estuary and Koggala lagoon) with respect to most of the studied characteristics. In another study by Quilang et al. (2007) who used multivariate analysis of morphometric data along with meristic characteristics to clarify the intra- and interpopulation variation in silver perch, *Leiopotherapon plumbeus* from three lakes in the Philippines, namely, Laguna de Bay, Taal Lake, and Sampaloc Lake. Results of cluster analysis showed that the specimens from Sampaloc Lake and Taal Lake were found in one group, while majority of the specimens from Binangonan and Tanay were in another cluster which suggest a closer morphological similarity between specimens from Sampaloc and Taal. A comparative study on the morphometric variation of *Sarotherodon melanotheron* (Ruppell) from brackish and fresh water habitats both in south-western Nigeria using morphometric and meristic characteristics showed that they were phenotypically separable populations of the same species (Omoniyi and Agbon 2007). The results revealed significant differences in body depth and caudal peduncle depth, which were suggested to have occurred as a result of difference in the temperature, salinity and substratum in the two water bodies. In another study of *T. trachurus* (Carangidae) among five different sampling locations in Turkey, Bektas and Belduz (2009) examined morphological variation by discriminant function analysis of 11 morphometric and 5 meristic characteristics which showed the heterogeneity between Aegean and Mediterranean populations. The Mediterranean population separated from all other and indicates two local populations for morphometric data, unlike meristic data that revealed no significant differentiation between two Mediterranean localities. Discriminant function analysis indicated that morphometric differentiation between the samples were largely due to differences in the head characteristics of the fish. The case studies of *T. trachurus* just reviewed illustrate how the morphometric characteristics are more powerful than meristic characteristics in discriminating the populations.

Truss Network Analysis produces a more systematic geometric characterization of fish shape than traditionally used dimensions for describing interspecific shape differences, because shape is more comprehensively described by truss networks (Humphries et al. 1981; Strauss and Bookstein 1982). To overcome the lack of knowledge on Truss based system, Turan (1999) briefly described a computerised approach to the collection of morphometric data taken with the Truss Network and different types of multivariate analyses to monitor patterns of differentiation among wild populations. In another study by Cadrin and Friedland (1999) who briefly reviewed the history of morphometric analysis with respect to fish stock discrimination and the developments of image analysis systems. Common protocols for sampling, analyzing, and interpreting variation associated with morphometric landmarks for stock identification applications was reviewed by Cadrin (2005).

Corti et al. (1988) used multivariate morphometry to investigate the distinctness and interrelationships of six stocks of the common carp (*Cyprinus carpio*) using truss network and a size component was clearly identified by multiple group principal component analysis. Canonical variate analysis computed only the shape components showed that the stocks were morphologically distinct and that the phonetic relationships based on allozymic and morphometric data are highly congruent. Chen et al. (1989) tried to discriminate cultivated and wild populations of the grey mullet (*Mugil cephalus*) using different univariate and multivariate clustering on 27 morphometric measurements. The results revealed that the mullets could be classified into three different groups: cultivated, semi-wild and wild populations. The results of the principal component analysis indicated that the most important characteristics to discriminate the above three groups are the snout length, eye diameter, the distance between the second dorsal and the anal fin, the length and height of the caudal peduncle. Tzeng (2004) used multivariate allometric coefficients and size-adjusted shape to elucidate the stock structure of spotted mackerel (*Scomber australasicus*) off Taiwan and concluded that there are three morphologically distinguishable stocks of spotted mackerel off Taiwan. Turan et al. (2004) investigated the status of populations of anchovies *Engraulis encrasicolus* collected from central (Sinop) and eastern (Trabzon) Black Sea, the Aegean Sea (Üzmir) and the eastern Mediterranean (Üskenderunusing morphometric characteristics. Plotting discriminant functions 1 and 2, explaining 93 % of between-group variability, revealed a high degree of dissimilarity among the anchovy samples, indicating that the anchovies in each sea represent different aggregations. The overall random assignment of individuals into their original group was high (80 %). Pairwise comparisons using multivariate analysis of variance (MANOVA) showed highly significant differences between all the samples ($P < 0.001$). Univariate analysis of variance (ANOVA) revealed significant differences with varying degrees between the means of the 4 samples for 16 out of 25 standardized morphometric measurements. Principal components analysis (PCA) indicated that the observed differences were mainly from the measurements taken from the head. Bagherian and

Rahmani (2009) studied morphological discrimination between two populations of shemaya, *Chalcalburnus chalcoides* (Actinopterygii, Cyprinidae), using a truss network. Truss distances between 15 landmarks of 66 specimens were measured. Size adjustment transformations were assessed by dividing characteristics (truss distances) by centroid size of specimen. Multivariate analysis of variance (MANOVA), principal component analysis and discrimination analysis were performed to investigate distinction and patterns of morphological variations between populations and sexes. The MANOVA (Wilks test) indicated a significant difference for mean vectors between populations and sexes. Discrimination analysis correctly classified 97 and 89.4 % samples to their original groups for population and sex, respectively. Janhunen et al. (2009) examined morphological variability among three geographically distinct Arctic charr (*S. alpinus* L.) populations reared in a common hatchery environment using 27 morphometric variables and most of the total variation was explained by the overall body robustness, dimensions of the head and caudal peduncle length. After controlling for a body size, significant heterogeneity in body shape was found among populations. Çakmak and Alp (2010) detected significant morphometric differences among the populations of Mesopotamian Spiny Eel, *Mastacembelus mastacembelus*, while meristic traits did not differ in three populations. Lower jaw length (LJL) was significantly smaller in Atatürk Reservoir population than the river populations of Tohma and Dicle. Stepwise discriminant analysis was applied for transformed morphometric and meristic data. In discriminant function analysis, morphometric differentiation was determined among the populations. The percentage of correctly classified individuals into their original groups was 71 % for Tigris and Tohma and 97 % for Karakaya populations.

Winans (1984) compared the conventional and truss network in juveniles of Chinook Salmon (*Oncorhynchus tshawytscha*) in an effort to study stock differences through shape change. He opined that the expression of morphometric covariability is influenced to some degree by the nature and timing of environmental variation during development. The concordance of the two results from the multivariate analyses on one genetic stock grown in two separate hatchery environments in his study suggested the presence of a distinct and desirable shape change in the early development of Chinook Salmon. The author further opined that the notion of using measures of multivariate morphometric variation to discern stock differentiation of juvenile. Hossain et al. (2010) also used both, morphometric characteristics and truss measurements to study variations of the endangered carp, kalibaus *Labeo calbasu*, from stocks of two isolated rivers, the Jamuna and Halda, and a hatchery. Significant differences were observed in four (maximum body height, pre-orbital length, peduncle length, and maxillary barbel length) of 12 morphometric measurements, and four (distance between insertion and origin of pelvic fin, distance between origin of dorsal fin and insertion of pelvic fin, distance between most posterior aspect of neurocranium and insertion of pelvic fin and distance between the tip of the snout and lower lip) of 22 truss network measurements among the stocks. For morphometric and landmark measurements, the 1st discriminant function (DF) accounted for 75.5 % and the 2nd DF accounted for 24.5 % of the among-group variability, and together they explained 100 % of the total among group variability. For the morphometric and truss network measurements, plotting DFs revealed high isolation of the stocks. The dendrogram based on morphometric and truss distance data placed the Jamuna and hatchery in one cluster and the Halda in another cluster, and the distance between the Halda and hatchery populations was the highest.

Applications to Indian freshwater and marine species

India has vast fishery resources with diverse species distribution that offers a large fish genetic pool with unmatched scope for genetic and evolutionary studies. However, in the recent years, the freshwater fish biodiversity in India are showing an alarming decline due to several factors and several species have been categorized as threatened in many parts of the country (Sarkar and Lakra 2010). This emphasizes an immediate need for initiating research and framing the strategies of actions for conservation and management techniques to protect these aquatic life forms. In the recent past, several studies have been made on the traditional morphometry however, studies based on truss based morphometry is still lacking.

Fernandez and Devraj (1989) and 1990 distinguished the stocks of two fish species *Coilia dussumieri* and *Harpondon nehereus* along the Northeast coast of India by morphometric characteristic sets using the analysis of covariance (ANCOVA) and discriminant analysis. Serajuddin (2004) reported that that the riverine population of spiny eel, *M. armatus* collected form Kalinadi, a tributary of Ganga river system at Aligarh contain two different stocks which differed in a number of morphometric characteristics. Saini et al. (2008) studied the comparative morphometrics of two populations of giant river catfish (*Mystus seenghala*) from the Indus river system in India. The stepwise discriminant analysis retained nine variables that significantly discriminated the Beas samples from the Sutlej samples. Using these variables, 91.2 % (original) and 89.0 % (cross validated) of fish were classified into their correct samples. Misclassification was higher for the Sutlej samples (12.5 %) than for the Beas samples (6.3 %). The results of the discriminant analyses showed that variability in the Beas samples was more homogeneous and provided a more characteristic picture of the group than the Sutlej samples. The univariate ANOVA revealed significant differences between the means of the two populations for 12 of the 28 transformed morphometric measurements. Sarkar et al. (2009) determined the stock identification of fish using morphometric and meristic methods collected from twelve different geographical locations and observed that the stocks were differed in the body structure, pectoral fins and mouth shape from most of the population but meristic characteristic was not much varied from each other.

Limited studies has been made based on truss based morphometry and confined only to marine ecosystems. Jayasankar et al. (2004) analyzed possible population differences in Indian mackerel (*Rastrelliger kanagurta*) from selected centers in the East and West coasts of India. Principal component analysis of truss landmark variables revealed that the area encompassing depth between the origin of anal and origin of second dorsal and caudal peduncle depth has high component loadings. Bivariate scatter plots of principal components showed a great degree of morphometric homogeneity between Indian mackerel populations from Mandapam, Kochi and Karwar. Gopikrishna et al. (2006) carried out Truss network analysis using data of the Asian seabass *Lates calcarifer* sampled from five different locations along the Indian coast to examine the stock difference. Principal Components Analysis was carried out on the multivariate morphometric data and observed that the first factor loadings were positive and nearly of the same magnitude indicating that PC I is a measure of size. A few truss measurements contributed to the shape differences among the stocks. As far as the shape differences are concerned, the Kakdwip stock was different from the rest of the stocks. A similar trend was discernible in the Chennai stock. However, there were no shape differences among the Chilka, Kakinada and Goa stocks. Sajina et al. (2011) studied populations of *M. cordyla* (horse mackerel) 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 using body shape morphometrics. A truss box method was followed, and 33 distance variables were extracted from digital images of sample specimens using the software platforms tpsDig2 and PAST. The transformed truss measurements were subjected to factor analysis and classification by cross-validation of discriminant analysis. Measurements from the anterior half of the fish body showed meaningful loadings on the first factor, and those from the caudal peduncle gave high loadings on the second factor. The combination of distance variables that produced the minimum amount of misclassification consisted of variables belonging to the middle portion of the body. Our results indicated a clear separation of the Bay of Bengal and Arabian Sea populations. The Mumbai and Cochin populations exhibited obvious mixing, indicating the possible existence of a unique stock along the west coast of India. In the cross-validation of the morphometrics by discriminant analysis, the most well-defined group was the Mandapam population, with only 3.59 % of the individuals being misclassified, followed by Digha, indicating limited gene flow in the Bay of Bengal populations. The strong morphometric differentiation observed between the Mandapam and Digha populations, in addition to the considerable coral reef features of the Gulf of Mannar region, suggests the existence of separate spawning stock populations of horse mackerel in these regions, which might require distinct stock assessment programs to provide effective management strategies for the east coast.

Conclusion

The present communication deals with a wide variety of methods used for the morphological differentiation, influential factors responsible for morphometric variation and also overview the morphometric variation among fish populations. It is evident that the morphometric landmark characteristics to identify phenotypic stocks is more than a century old, the development of truss based system with the advances in analytical methods revolutionized the study of morphometric variation which have increased the power of morphometric analysis for stock identification. Since the quantitative morphometric characteristics are generally related to fitness and respond to natural selection thus the local adaptation, rapid adaptive divergence between recently separated groups, and genetic differences maintained by selection in the face of gene flow may all be reflected in these traits. Moreover, the environmental as well as genetic influences which are responsible for the phenotypic variation can be identified by adopting a 'holistic' approach through employing a broad spectrum of complimentary techniques. However, despite its importance in the development of fishery management, stock identification continues to be an afterthought.

As a potential indicator of phenotypic stocks, analysis of morphometric landmarks is a valuable tool that complements other stock identification methods. The identification, discrimination, and delineation of phenotypic stocks are essential for population modeling, which generally assumes homogenous ontogenetic rates within a stock. Our review on these aspects agreed that the broad strategies for stock identification are to incorporate results from the different methods and disciplines in order to achieve conclusions about population structure consistent with various approaches. Globally, in the recent years the morphometric techniques are boosting the utility of morphometric based research in fish stock identification to facilitate the sustainable utilization of fishery resources and biodiversity conservation. Unfortunately in India, the conservation and management of the rich fish diversity is ultimately not much focused in this perspective, which therefore, demands for the identification of suitable stocks for fishery management. Initiative has been taken recently at National Bureau of Fish Genetic Resources (Indian Council of Agricultural Research), to identify the stock structure of selected prioritized fish under network mode using truss based morphometry and molecular tools (Lakra and Sarkar 2010). It is expected that this study will be helpful in order to accomplish the knowledge based on diversified morphometry techniques and to improve the quality of stock identification research.

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