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A Note on The Examination of Morphometric Differentiation Among Fish Populations: The Truss System

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Abstract: Identifying intraspecific units or stocks of a species with unique morphological characters enables a better management of these subunits of species and ensurs perpetuations of the resources. Multivariate morphometry has been commonly used to investigate the discreteness and interrelationships of stocks within a species. Different types of body measurements have been traditionally used to charecterise stocks. However these measurements have recently been criticised because there are several biases and weaknesses inherent in traditional characters. As an alternative, a new system of morphometric measurements called The Truss Network System has been increasingly used for stock identification. In this review a computer-originated approach to the collection and analysis of morphometric characteristics of stocks is described.

Key Words: Stock identification, multivariate morphometric investigation, the Truss System.

Balık Populasyonları Arasındaki Morfolojik Farklılaşmanın İncelenmesi Üzerine Bir Not: Truss Sistemi

Özet: Bir balık türünün morfolojik karekterler bakımından farklı stoklarının belirlenmesi bu türün alt birimlerin daha iyi bir şekilde yönetilmesine imkan verir ve bu kaynakların devamını sağlar. Çokdeğişkenli morfometri bir türün içerisindeki stoklar arasındaki ilişki ve farklılığın araştırılmasında yaygın olarak kullanılmaktadır. Farklı vücut ölçümleri öteden beri geleneksel olarak stokları karakterize etmek için kullanılmaktadır. Fakat bu geleneksel karakterler ötedenberi gelen bazı zayıf ve meyilli özelliklerinden dolayı eleştirilmeye başlanmıştır. Alternatif olarak Truss yöntemi denen yeni bir morfometrik ölçüm sistemi stokların tanımlanmaları için gittikçe yaygınlaşarak kullanılmaktadır. Bu derlemede morfometrik karakterlerin toplanması ve analizi için bilgisayar kaynaklı bir yöntem tanıtılmaktadır.

Anahtar Sözcükler: Stok belirleme, çok değişkenli morfometrik araştırma, Truss Sistemi

Introduction

Morphological characters have been commonly used in fisheries biology to measure discreteness and relationships among various taxonomic categories. There are many well documented morphometric studies which provide evidence for stock discreteness (1, 2, 3, 4, 5, 6, 7). However, the major limitation of morphological characters at the intra-specific level is that phenotypic variation is not directly under genetic control but subjected to environmental modification (8). Phenotypic plasticity of fish allows them to respond adaptively to environmental change by modification in their physiology and behaviour which leads to changes in their morphology, reproduction or survival that mitigate the effects of environmental variation (9, 10). Such phenotypic adaptations do not necessarily result in genetic changes in the population (11, 12), and thus the detection of such phenotypic differences among populations cannot usually be taken as evidence of genetic differentiation. For example, Swain et al. (13) used the truss system in identification of hatchery and wild populations of Coho salmon (*Oncorhynchus kisutch*). They found significant variation, which was attributed to the rearing environment rather than to genetic differences between hatchery or wild populations.

Environmentally induced phenotypic variation, however, may have advantages in the stock identification, especially when the time is insufficient for significant genetic differentiation to accumulate among populations. Due to random genetic drift, genetic differentiation may occur very slowly in the typically large population of commercial marine fishes (14). Genetic markers are generally oversensitive to a low level of gene flow: a relatively low level of exchange between stocks, negligible from a management perspective, may be sufficient to ensure genetic homogeneity (15, 16, 17). Therefore, molecular markers may not be sufficient to detect existing genetic variation among populations, and also only a small proportion of DNA is analysed by molecular markers. However, phenotypic markers may detect morphological differentiation due to environmental differences in the habitats of partially-isolated stocks, which may be a practical level of partitioning among self-recruiting stocks. Such self-recruiting stocks may react independently to exploitation (16), even without showing genetic differentiation. Morphometric and meristic analysis can thus be a first step in investigating the stock structure of species with large population sizes.

Morphometric studies are based on a set of measurements which represent size and shape variation and are continuous data, in contrast to meristic characters, which are discrete or noncontinous data. Therefore, the predictive abilities of morphometric and meristic characters differ statistically and are likely to be lower for the meristic characters (11). Thus, morphometric data should be analysed separately from meristic data in multivariate analyses (11).

For the past 50 years, morphometric investigations have been based on a set of traditional measurements described by Hubbs & Lagler (18). These measurements have recently been criticised because they are concentrated along the body axis with only sampling from depth and breadth, and most measurements are in the head. 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.

The results of a morphometric analysis can depend on the particular set of measurements chosen. 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 (19). Thus these traditional measurements represent a biased coverage of body form (19), and success in selecting effective characters has been attributed to a matter of chance (20).

As an alternative, a new system of morphometric measurements called the Truss Network System (18) has been increasingly used for species and especially for stock differentiation (2, 6, 7, 13, 20, 21, 22, 23). The Truss Network System covers the entire fish in a uniform network, and theoretically should increase the likelihood of extracting morphometric differences within and between species. A regionally unbiased network of morphometric measurements over the two-dimensional outline of a fish should give more information about local body differences than a conventional set of measurements (19, 20). There is evidence that the Truss Method is much more powerful in describing morphological variation between closely related fish taxa (e.g. stock) than traditional measurements (2, 19, 20).

The main objective of this article is first to describe a computerised approach to the collection of morphometric data taken with the Truss Network, and second to give types of multivariate analyses to monitor patterns of differentiation among wild populations.

Laboratory procedure

Truss network measurements are a series of measurements calculated between landmarks that form a regular pattern of connected quadrilaterals or cells across the body form. Cells and truss characters are referenced according to the scheme of Strauss & Bookstein (19); for example, the distance between landmarks a and b is a truss character in first quadrilateral or cell (landmarks a, b, k, l) (Figure 1). Measurements of specimens are made by collecting X-Y coordinate data for relevant morphological features, and followed the three-step process as described below.

Positioning

Fish are placed on acetate sheets, and body posture and fins are teased into a natural position. A block of expanded polystyrene (2 cm) can be placed beneath the acetate sheet to facilitate pinning.

Pinning

Morphological features or landmarks that are distinctive and homologous from specimen to specimen are selected around the outline of the fish form. Each landmark is marked by piercing the acetate sheet with a dissecting needle. The twelve landmarks produced for herring (7) are illustrated in Figure 1. Measurements should be made on one side of the each specimen throughout sampling. Additional data, such as eye diameter and head width can also be recorded and added in the truss data.

Digitizing

The acetate sheet is placed on a light box, and a camera, connected to a monitor, video and computer, is set at the top of the light box, and the image is stored on the screen of the monitor to view interlandmark distances. The X-Y coordinate value for the positions of landmarks is digitized for each fish by Measurement TV Program (Data Crunch Product). Alternatively, an X-Y coordinate digitizing pad can also be used to establish a reference set of X and Y axes to view interlandmark distances. All measurements are transferred to a spreadsheet file (e.g. Excel or Lotus), and X-Y coordinate data is transformed into linear distances by computer (using the Pythagorean theorem) for subsequent analysis.

Data analysis

When all samples for all populations have been taken from the same ages there is no need to eliminate the size effect in the data set. Otherwise, an important stage in the data preparation for morphometric analyses is to eliminate any size effect in the data set when comparing fish of different sizes. Variation should be attributable to body shape differences, and not related to the relative size of the fish. Therefore, transformation of absolute measurements to size-independent shape variables is the first step of the analyses. Several transformation methods previously shown to be effective in removing such size-dependent variation can be compared (24). The transformations are:

I) RATIO: $M_{adj}=M/SL$, ie. division by standard length.

II) LGRATIO: $M_{adj}=\log M/\log SL$ = the log of ratio

III)ALLOM1: $M_{adj}=\log M-\beta_1(\log SL-\log SL_{mean})$

IV)ALLOM2: $M_{adj}=\log M-\beta_2(\log SL-\log SL_{mean})$

Where:

M: original truss measurement

M_{adj}: size adjusted truss measurement

SL: standard length of fish

SL_{mean}: overall mean of standard length

β_1: coefficient of the overall linear regression of logM against logSL

β_2: average pooled within-sample coefficient of the regressions of log M against log SL

Base-10 logarithms are used for all variables. Standard length is used for all cases, since it correlates strongly with other morphometric characters (24, 25, 26). 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. Standard length (landmark distance between a and f, Figure 1) must be excluded from the analyses. The second way of eliminating the size effect in principal components analysis (PCA) is to eliminate the first principal component (PC) from the analysis, 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 (27), though weight is a power of length (28). In this way, each morphometric measurement is divided by the individual gutted weight prior to multivariate analysis.

Morphological differentiation may vary between the sexes in some fish species: Creech (1993) reported greater variation between two sandsmelt species in females than in males. 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 should be standardised to a 0 mean and a standard deviation of 1 and can be submitted to a principal components analysis and a multiple-discriminant function analysis (DFA) or canonical analysis (CA) using a statistical package program (e.g. SPSS, SYSTAT) and graphs can also be generated using these programs. The transformed data are also used for other statistical analyses (e.g. Analysis of Variance (ANOVA), and Multivariate Analysis of Variance (MANOVA)).

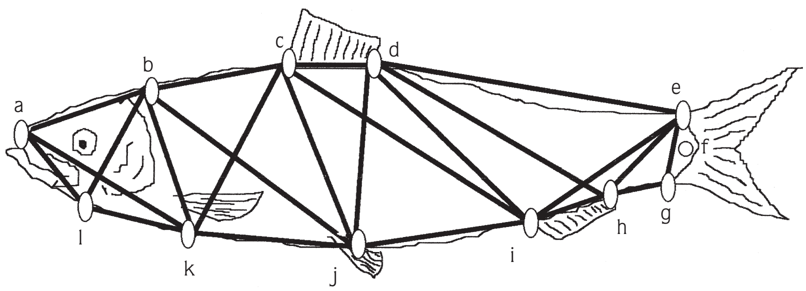

Figure 1. Locations of the 12 landmarks for constructing the truss network on fish illustrated as open circles and morphometric distance measures between circles as lines. Landmarks refer to (a) anterior tip of snout at upper jaw, (b) most posterior aspect of neurocranium (beginning of scaled nape), (c) origin of dorsal fin, (d) insertion of dorsal fin, (e) anterior attachment of dorsal membrane from caudal fin, (f) posterior end of vertebrae column, (g) anterior attachment of ventral membrane from caudal fin, (h) insertion of anal fin, (i) origin of anal fin, (j) insertion of pelvic fin, (k) insertion of pectoral fin, (l) posteriomost point of maxillary. For landmarks b, c, d, h, i, j, k, l, points were made at their respective positions at the closest point to the body on a line perpendicular to the horizontal axis of the fish. Taken from Turan (7).

Multivariate analyses

Multivariate techniques simultaneously consider the variation in several characters and thereby assess the similarities between samples. 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. 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.

CA is used to discriminate the samples according to the variables. CA 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. The percentage of correctly classified individuals gives a measure of the morphological distinctness of the samples.

Principal components and canonical analysis can be used to produce graphs to visualise relationships among the individuals of groups by plotting population centroids of 95% confidence ellipses of first two canonical functions (CFs) and PCs The measurements with high loadings in CA are between-sample diversity, and hence differ from those in PCA (which have total diversity). Each principal component contains the percentage of total variance of all variables. But in CA, each function contains the percentage of the total between-groups variability. Therefore, CA is used to describe the pattern of phenotypic differentiation among samples.

Univariate analysis of variance can be used to compare the variation among samples for size-adjusted truss measurements. *Post hoc* multiple comparison tests can also be performed to find the number of significant morphometric characters between pairs of samples. The number of significantly different measurements among groups is an additional indication of the degree of group separation. The effect of sex on the truss measurements should also be tested using univariate statistics.

Multivariate analysis of variance can be performed to test the significance of differences among the samples in the data set.

Conclusions

The Truss System can be successfully used to investigate stock separation within a species that allows, in a long term, a better and direct comparison of morphological evolution of stocks, while using the same set of measurements. The particular usefulness of the Truss System as a fisheries management tool is that it is capable of examining large numbers of samples in a short time. It is also effective in identification of stocks and improving the biological basis of management, especially when they are used in conjunction with molecular genetic markers (7). The major drawback of this technique is that it requires computer image processing equipment to perform the analysis.

The system of measurement and analysis described is based on two premises: first, that collections of anatomical points and the distance measures among them must be homologous from form to form, because only the biological homology of two configurations makes meaningful their scientific description and comparison. In addition the unbiased network of morphometric measurements over the whole body removes the need to find the types of characters and optimal number of characters for stock separation, and provides information over the entire fish form. Secondly, an adequate collection of measured distances should at least permit the reconstruction of the configuration of landmarks, since information is otherwise lost. As a result it could be concluded that the Truss Network is a geometric protocol which fulfills these requirements.

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