Chapter 9
Stock Identification of Marine Populations

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Idea and Aims

This chapter is intended to introduce researchers to an application of morphometric analysis that examines population structure of marine species. The approach has been effective for identifying the appropriate spatial scale for resource management units that reflect the underlying spatial patterns in the population. Several examples of stock identification of fishery resources are provided.

Introduction

Morphometric analysis is a valuable tool for scientists involved in studying marine populations, because it helps to identify intraspecific groups of animals that can be effectively monitored and conserved. Among a wide variety of methodological approaches, geographic patterns in morphology provide a unique perspective on spatial population structure. Conventions for sampling, analysis and interpretation have been developed to promote representative and meaningful conclusions, and many case studies have demonstrated the value of morphometric analysis for stock identification and resource conservation.

Management of living marine resources depends on accurate stock identification. Recognizing distinct subpopulations within a species is necessary to evaluate population trends and productivity as well as sustainability of human impacts. Population dynamics are more easily modeled and monitored if the appropriate "unit stock" is defined so that each of the components of biological production (growth, mortality and reproduction; Russell 1931) is determined from within the group rather than from outside the group. For example, new individuals enter a unit stock primarily from reproduction within the stock rather than from migrants from an adjacent

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stock. Self-sustaining stocks are the units that resource managers need to recognize to meet their conservation and sustainability objectives.

Stock identification is a critical requirement of both conservation biology and fisheries science. Although the two fields have different objectives, they both involve inferences about how populations respond to perturbations or restoration efforts. Fishery management is usually focused on maintaining maximum sustainable yield (MSY; Mace 2001). Determination of MSY is most appropriate for demographically independent units that are essentially isolated from other groups on ecological time scales (i.e., years to decades). Conservation biology considers both long-term and short-term dynamics to determine the appropriate stock units to manage. Evaluating the risk of extinction for a species involves the recognition of "evolutionarily significant units" (Waples 1991) that are reproductively isolated over geologic time scales (i.e., millennia) and have developed unique adaptations. However, recovery of threatened species involves restoration of smaller, demographically independent management units that are self-sustaining on ecological time scales. Therefore, identification of unit stocks that are isolated from other stocks and maintain homogeneous vital rates is essential for management of living marine resources, whether for conservation biology or fishery management (Eagle et al. 2008).

Conservation biologists and fishery scientists approach the identification of stocks from many methodological perspectives to attain a holistic view of reproductive isolation and homogeneity (Begg and Waldman 1999). Stock identification has developed into an interdisciplinary synthesis that involves geographic distribution, movement among areas and geographic variation in genetics or phenotypic traits (Cadrin et al. 2005). Morphological variation is phenotypic (i.e., it is influenced by both genetic composition and environmental factors), and heritability of morphometric characters is generally low to moderate (Swain et al. 2005). Therefore, temporal stability in geographic variation of morphology is essential for stock identification applications, so that stocks are not determined on the basis of ephemeral differences in the environment. Despite the influence of environmental factors on morphological variation, patterns of variation can indicate groups that are isolated enough to maintain phenotypic differences. Morphometric patterns are also often associated with geographic differences in growth, maturity, or mortality which are critical to population dynamics. Adaptive significance of morphological features can add powerful interpretability to patterns of variation. For example, body form, fin size and fin location are adaptive for movement and maneuverability of fishes (Webb 1984) and cetaceans (Fish 1998); Position of the mouth and head shape are associated with trophic ecology (e.g., Costa and Cataudella 2007); Abdomen size reflects energetic investment, feeding and spawning condition (e.g., Armstrong and Cadrin 2001); Body armor (e.g., scutes, spines) can indicate different predatory environments (e.g., Walker 1996); and secondary sex characters (e.g., dorsal humps and fin size of fishes; chelipeds of crustaceans) can indicate behavioral differences and size at maturity (Cadrin 2000).

Morphometric analysis offers a unique perspective to the investigation of population structure. Patterns in morphology have been used to identify and discriminate stocks for nearly a century (Teissier 1936). More recently, morphometric patterns

are considered in the context of information on genetic variation and movement patterns for an interdisciplinary analysis of population structure.

Methods

Conventional protocols for sampling and analyzing morphometric data have been developed for a wide range of applications. Methods for morphometric stock identification reflect general conventions, and additional features should be considered that are more specific to investigating stock structure.

Sampling Designs

Stock identification typically progresses in stages, from exploratory to confirmatory, and is finally applied as either classification or mixture analysis. Each stage has different sampling requirements:

Exploratory Stock Identification – When little is known about stock structure of a species, general information on geographic distribution (e.g., research surveys, fishing grounds) should be used to define putative stocks, and each of them should be sampled to explore general patterns of variability throughout the range of the species. At this stage, wide geographic coverage of samples across areas and seasons is more important than sample sizes within groups, because groups have not been defined. Optimal sample sizes should be based on stability of pooled-group multivariate analyses (e.g., there should be at least three times as many samples as variables; Saila and Martin 1987). At the exploratory stage, data collection should include as many potentially explanatory variables as possible (e.g., age, gender, maturity stage, depth, color) to be considered as potential factors to be included in analysis of morphometric patterns. As with any study, a comprehensive literature review is valuable at the exploratory stage to design sampling.

Confirmatory Stock Identification – Once a stock structure hypothesis has been developed, either by exploratory analysis of morphometric variation or information from other approaches to stock identification, "baseline samples" from each putative stock should be collected in areas and at a time of year when stock mixing is minimal (e.g., during the spawning season, on spawning grounds). Sample sizes within groups should be a minimum of 50 specimens to derive reliable estimates of covariance among features (Tabachnick and Fidell 1989), recognizing that sexual dimorphism may require separate-sex analyses. A critical aspect of sampling design for confirmatory analysis is to include all putative stocks of interest. Including an "out-group" (i.e., another group far from the area of interest) can provide context in which to understand the magnitude of variation among the more related groups being studied.

Stock Discrimination – Once substantial and meaningful differences are confirmed to exist among stocks, those differences can be used to classify individuals to a stock. Accordingly, areas between the source samples used for confirmatory

analysis (i.e., areas and seasons with possible mixed stocks) should be representatively sampled. A statistically significant difference among baseline samples is essential, but not always enough, for accurate classification. Although there is no rule of thumb to determine the magnitude of difference needed for accurate discrimination, there are useful diagnostics to judge performance (e.g., extrinsic classification or cross-validation). Stock discrimination can be used to help delineate geographic (and possibly seasonal) boundaries among stocks or to determine stock composition in a mixture (e.g., a mixed-stock fishery).

Processing Specimens

Many aspects of image processing for stock identification are similar to other applications of morphometric analysis. For example, images must be calibrated, measurement error should be calculated over repeated measures, and shape change associated with preservation method should be assessed. However, the choice of morphological features for possible stock identification characters may require additional consideration.

Most of the recent advances in morphometrics have been focused on landmarks (e.g., Elewa 2004), and landmark methods comprise a large portion of the research in morphometric stock identification. However, many studies use other aspects of morphology to study stock structure such as outline features, circuli patterns and meristics. All four categories of features can be measured using image analysis.

Landmark features can be used on any morphological feature (e.g., bones, otoliths), but are generally used to measure general body form. Some landmark features that are associated with biologically meaningful variation are fin placement, body depth, caudal depth, head size, position of the mouth, and eye position. Most of these features are captured by Straus and Bookstein's (1982) box-truss network, which was initially developed for fishes, but is applicable for diverse taxa. Although the box-truss was initially developed for traditional multivariate analysis of box-truss distances, the box-truss landmarks are also valuable for geometric analysis, because they represent an efficient measure of general body form that is related to function. An augmented set of triangle-truss landmarks and linear distances that capture general body form, fin size, mouth size and eye position is illustrated in Fig. 9.1.

Outline features have also been extensively applied to stock identification. Outlines of hard parts such as otoliths (i.e., "ear bones") or scales are typically used to investigate geographic variation of bony fishes, but perhaps the most biologically meaningful patterns of variation in outline shape have been found for bivalves. For example, ontogenetic stages of Atlantic sea scallop (*Placopecten magellanicus*) are reflected in outline shape of their valves (Dadswell and Weihs 1990). After juvenile scallops detach themselves from their byssal threads, they are active swimmers until they become more sedentary adults. During the juvenile, swimming phase, the valve hinge becomes more prominent, and the valve (shell) becomes more circular, both of which confer greater swimming velocity and duration; during the adult, sedentary phase, the valve becomes wider and more oval (Fig. 9.2, C. O'Keefe SMAST video

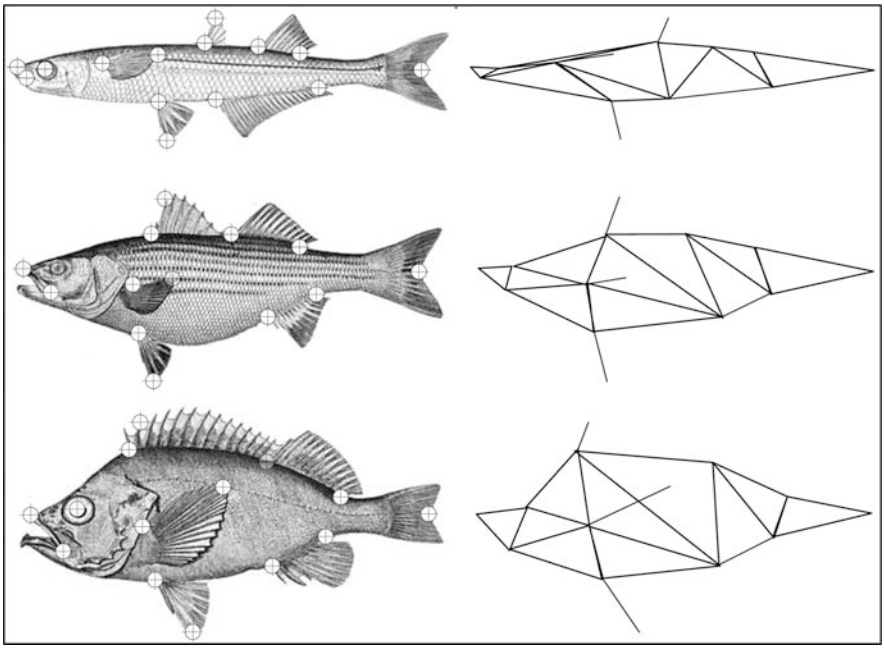

Fig. 9.1 Augmented triangle truss networks of landmarks ("targets" on fish images) and distances (line segments on *right*) for Atlantic silverside (*Menidia menidia*), striped bass (*Morone saxatilis*) and Acadian redfish (*Sebastes fasciatus*); fish drawings from Bigelow and Schroeder (1953)

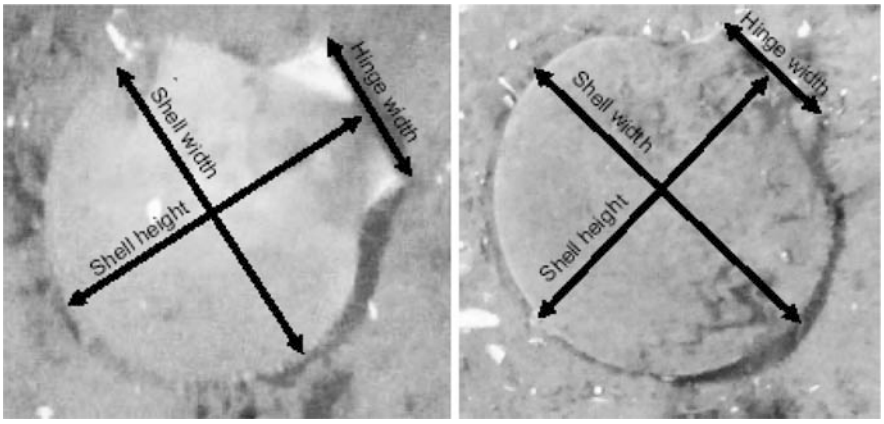

Fig. 9.2 Morphometry of sea scallop (*Placopecten magellanicus*) shells. Note that the juvenile *(left)* has nearly equal shell height and shell width and a wider relative hinge width than the adult *(right)* which is has greater shell width than shell height (images form C. O'Keefe, SMAST video survey)

survey). Kenchington and Full (1994) quantified these ontogenetic shape changes using Fourier analysis of valve outlines to discriminate four scallop beds. The geographic variation in valve shape was associated with differences in age at maturity and swimming behavior.

Landmark and outline features are commonly used characters for morphometric analysis and morphometric stock identification. Two other aspects of morphology, circuli patterns and meristics, have also been useful for stock identification. Circuli patterns are growth rings in the hard parts. Otoliths of bony fishes are used for a wide range of applications to identify annuli (i.e., yearly bands) to determine age, to identify daily growth rings to determine hatch date, and to investigate differences in growth rates among groups. For example, relative growth rates of Atlantic salmon (*Salmo salar*) can be measured from circuli patterns on scales (Fig. 9.3, F. Hogan,

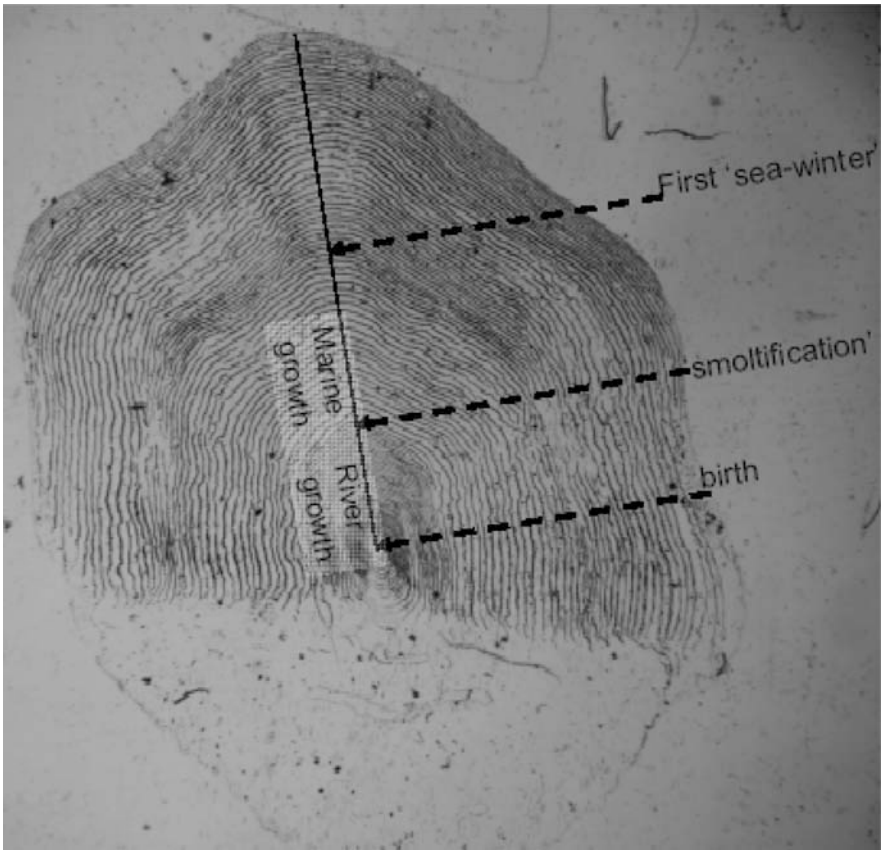

Fig. 9.3 Image of a scale from Atlantic salmon (*Salmo salar*). The solid line is a 'transect' from the birthmark to the scale margin, *arrows* indicate ontogenetic stages: birth, 'smoltification' (i.e., the transition form river to marine environments), and the first winter in the marine environment (image form F. Hogan, NOAA/UMass CMER Program)

University of Massachusetts) and compared among stocks. The advantage of using circuli patterns to study stock structure is that they are directly related to growth rate, a phenotypic character that is critical in modeling population dynamics and sustainability.

Meristics are discrete morphological features that can be counted, rather than measured (e.g., vertebrae, fin rays, gill rakers). Meristic characters have been used extensively for stock identification as a phenotypic character that is typically influenced by early-life history environments. Merisitic values (i.e., the number of discrete parts) are often determined during a distinct ontogenetic stage when the features are developing and sensitive to environmental influences. For example, the number of fish vertebrae is determined during the larval period, and is usually inversely related to temperature (Lindsey 1988).

Similar to the way morphometric analysis of landmarks and outlines have benefited from technological developments in image analysis, the study of circuli patterns and meristics have advanced in recent decades. Circuli patterns, including the number of circuli and circuli spacing, can be measured using luminescence transects from the focus or center of the growth structure to the margin (Fig. 9.3). Meristics can also be counted via imaging.

All four categories of features (landmarks, outlines, circuli or meristics) rely on thoughtful choice of characters to efficiently represent morphology with the fewest number of characters. There are an infinite number of morphometric features that can be measured, many of which are redundant, some of which are critical for capturing meaningful variation among stocks. Homology of characters (i.e., the same developmental feature among specimens; Strauss and Bookstein 1982) is critical for standardization and supports biological interpretations. Measurement of characters must also be repeatable among specimens, researchers and methodologies. Finally, prior information on life history and morphological patterns in related species should be considered in the choice of characters to measure.

Statistical Analysis

Morphometric data are multivariate in the strictest sense: several to many variables are needed to measure morphometry, and each variable is structurally correlated with others. All dimensions increase with size, and shape variation is a contrast in relative growth. Therefore, all categories of morphometric data are analyzed using multivariate analysis.

A simple example of a hypothetical sample of nine lumpfish (*Cyclopterus lumpus*) specimens is illustrated in Fig. 9.4 to demonstrate how morphometric data can be analyzed using multivariate analysis. The nine lumpfish represent a range of sizes and shapes that are crudely measured using a simple box truss network of six distances.

The data measured from the hypothetical lumpfish are listed in Table 9.1 as raw distances (cm) and natural log transformed distances. Log transformation is typical in morphometric analysis for statistical reasons (bivariate relationships are more

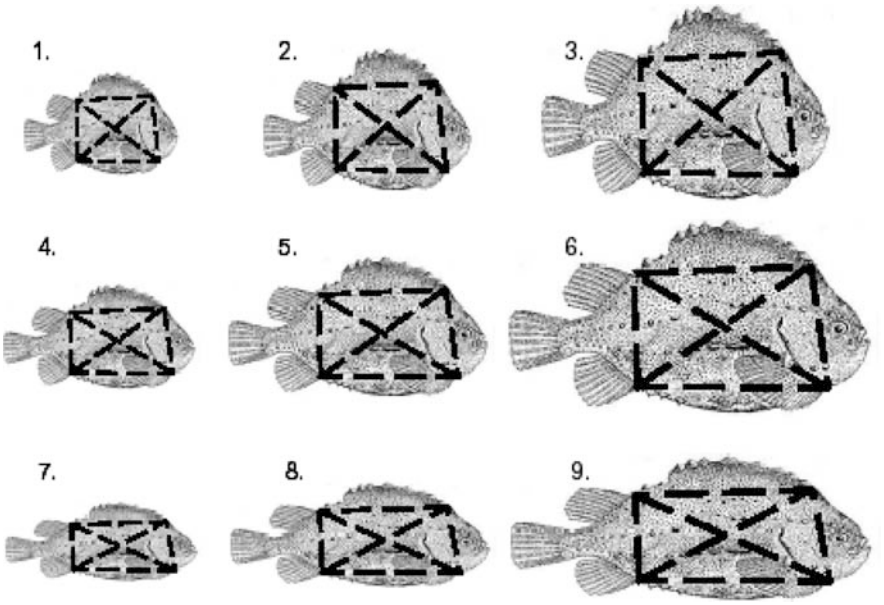

Fig. 9.4 Hypothetical sample of nine lumpfish, with a simple box-truss network of linear morphometric distances

linear, variances are more homogeneous) as well as theoretical reasons (distances increase by multiplicative growth; Huxley 1932). Note that log transformed data have more similar arithmetic means and standard deviations than the untransformed data. The six variables measured from the lumpfish specimens are highly correlated (Fig. 9.5).

Principle components analysis (PCA) shows that the greatest source of variation and covariation in the data is related to size (Table 9.2), because all six variables "load positively" on the first principle component (i.e., each variable is positively related to a composite variable that explains the most variation in the data). The second component of variance is a shape component, because it contrasts body depth (i.e., head depth and caudal depth load strongly positive) and body length (i.e., dorsal length and ventral length load strongly negative; diagonal distances load moderately negative). Therefore, most variance in the data (PC1) is from size differences among specimens, and the greatest variance in shape (PC2) is differences in relative depth.

The distribution of PC scores for each specimen also shows that large fish have positive PC1 scores, and small fish have negative PC1 scores (Fig. 9.6). The shape axis (PC2) clearly distinguishes deep and short fish (those with positive PC2 scores) from shallow and long fish (those with negative PC2 scores). There are three groups of fish with different shape: deep-short, intermediate and shallow-long; each group has three specimens: small, intermediate and large.

This simple example shows how multivariate analysis can examine patterns of variation for several correlated variables by deriving two composite variables that

Table 9.1 Six linear distances measured from nine hypothetical lumpfish (untransformed data in cm, above; log transformed data below)

Specimen	Head depth	Caudal depth	Dorsal length	Ventral length	Diagonal 1	Diagonal 2
1	9	8	12	13	20	18
2	16	14	20	22	31	29
3	26	23	31	34	46	43
4	9	8	17	20	25	23
5	16	14	27	30	37	35
6	26	23	42	45	56	52
7	5	4	17	20	23	20
8	11	8	27	30	35	31
9	17	15	42	45	52	46
Mean	15.0	13.0	26.1	28.8	36.1	33.0
Std.dev.	7.4	6.7	10.8	11.2	12.9	12.0
1	2.20	2.08	2.48	2.56	3.00	2.89
2	2.77	2.64	3.00	3.09	3.43	3.37
3	3.26	3.14	3.43	3.53	3.83	3.76
4	2.20	2.08	2.83	3.00	3.22	3.14
5	2.77	2.64	3.30	3.40	3.61	3.56
6	3.26	3.14	3.74	3.81	4.03	3.95
7	1.61	1.39	2.83	3.00	3.14	3.00
8	2.40	2.08	3.30	3.40	3.56	3.43
9	2.83	2.71	3.74	3.81	3.95	3.83
Mean	2.59	2.43	3.18	3.29	3.53	3.44
Std.dev.	0.54	0.57	0.43	0.41	0.37	0.38

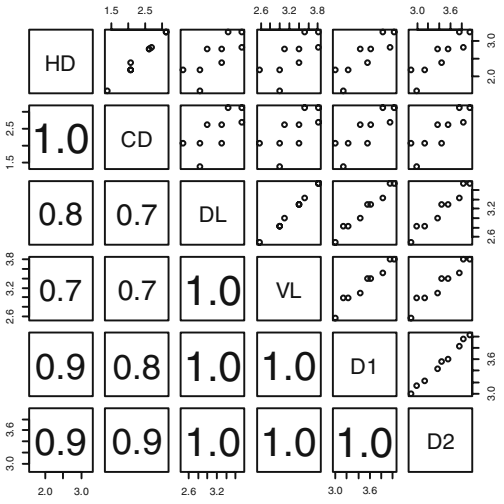

Fig. 9.5 Bivariate relationships among the six box-truss variables measured from the lumpfish sample. Data are natural log transformed. Variable codes are on the diagonal, bivariate distributions are above the diagonal and correlation coefficients are *below*

Table 9.2 Principle component (PC) loadings for six linear distances measured from nine hypothetical lumpfish (data in Table 9.1)

Component	Head depth	Caudal depth	Dorsal length	Ventral length	Diagonal 1	Diagonal 2
PC1	0.490	0.509	0.374	0.351	0.337	0.352
PC2	0.427	0.537	– 0.458	– 0.484	– 0.232	– 0.180

can be more simply interpreted. For morphometric stock identification, the first principle component of variance is typically removed to derive size-free shape discrimination among groups (e.g., Burnaby 1966). If the deep-short lumpfish were from one putative stock, and shallow-long fish were from another, a size-adjusted discriminant function would be similar to PC2.

Once a discriminant function has been developed from a sample of known group membership (i.e., "baseline samples"), the function can be used to classify individual specimens to a stock (i.e., "mixture samples"). One measure of classification accuracy is the ability to classify baseline samples, but this intrinsic classification tends to overestimate accuracy, because it is based on the same specimens that are used to establish discriminant functions. A more reliable measure of accuracy is

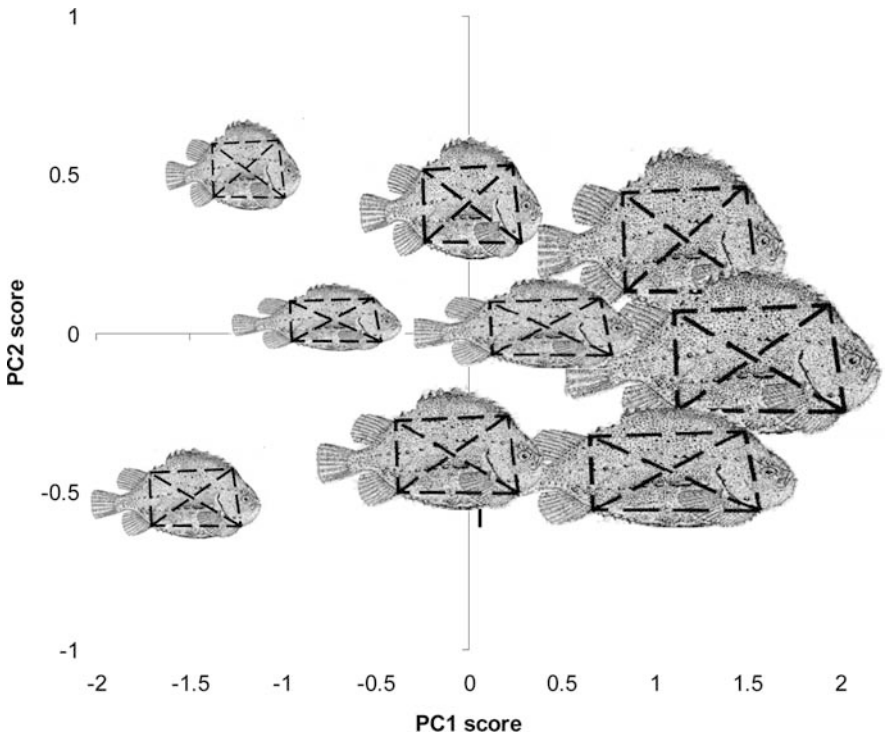

Fig. 9.6 Principle component scores of the nine lumpfish specimens (images age centered on the specimen's score), with PC1 serving as an index of size, and PC2 an index of relative body depth

extrinsic classification, or cross-validation, which uses known-stock individuals that are not in the baseline sample to verify accurate classification. Discriminant scores can be used to delineate stock boundaries or to determine the composition of each stock in a mixture. For example, stock composition can be determined for the catch of a fishery that exploits a mixture of spawning groups during the feeding season.

The lumpfish example above used traditional linear distances, but data from outlines (e.g., amplitudes from a series of Fourier harmonics) or circuli analyses (e.g., distances between successive circuli) can be similarly analyzed with PCA and discriminant analysis. One disadvantage of multivariate analysis of traditional linear distances is that it ignores the relative geometry of dimensions (e.g., head depth and dorsal length of lumpfish share a landmark). Geometric analysis of landmarks retains the relative position of landmarks and quantifies deformations from one set of landmarks to another. For example, Fig. 9.7 depicts the flattening needed to

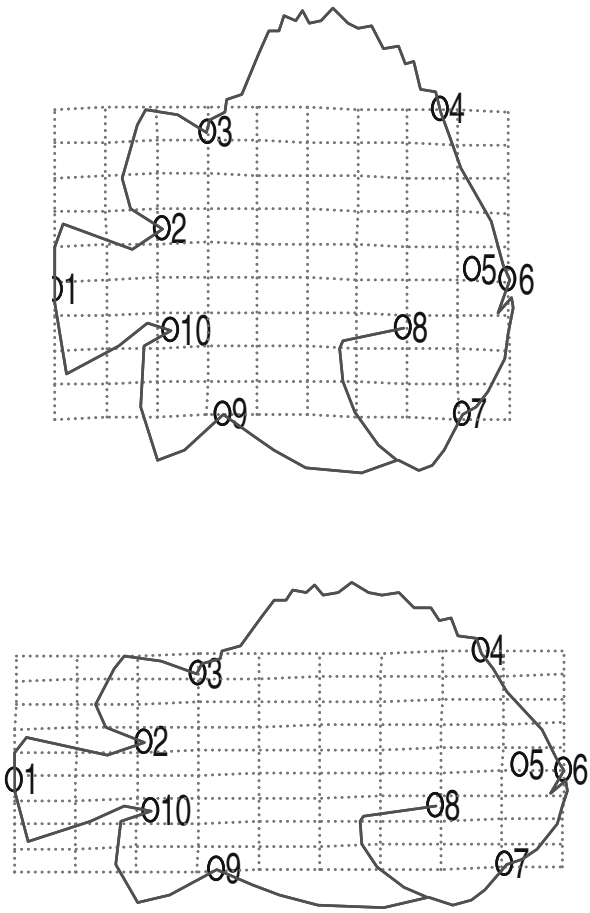

Fig. 9.7 Deformation from lumpfish specimen 1 to specimen 7, as measured by thin plate spline analysis (landmarks are numbered, the outline and grid are overlaid to help interpret shape variation)

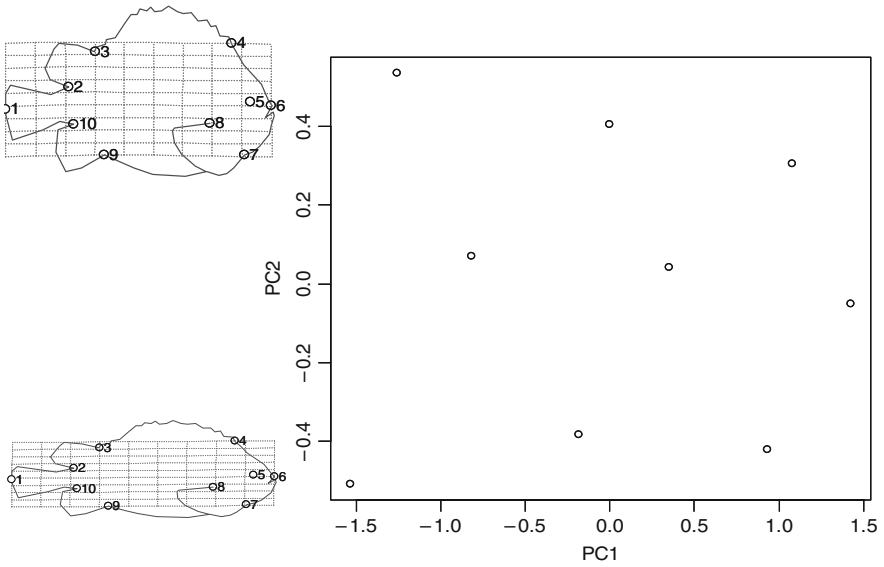

Fig. 9.8 Synthetic approach of traditional multivariate analysis of linear box-truss distances (PC score plot on *right*) and geometric analysis of landmarks (fish images on *left*), as illustrated using PC2 of the lumpfish data, which is associated with a general flattening

transform lumpfish specimen 1 to specimen 7. Similar to traditional distances, geometric deformations can also be used for morphometric stock identification.

Several recent case studies of morphometric stock identification have combined traditional analyses of linear distances with geometric analysis of landmarks (Cadrin and Silva 2005; Sheehan et al. 2005). The synthetic approach involves multivariate analysis of linear dimensions and geometric analysis of extreme specimens to illustrate the deformations associated with components of variation or differences among groups. For example, the geometric analysis of the extreme PC2 scores from the lumpfish data shows that PC2 is associated with a flattening (Fig. 9.8).

Conclusions

Morphometric analysis provides a complementary tool for stock identification. It can reveal patterns of life history that vary among subpopulations, indicating limited movement between groups and possible genetic variation. Therefore, in concert with information on genetic isolation, movement patterns and other phenotypic variation, morphometry has a distinct role in stock identification of marine populations. One of the strengths of morphometric stock identification is that patterns of geographic variation can be interpreted in terms of adaptive characters that influence population dynamics. Thoughtful interpretation of morphometric patterns confers meaning and emphasizes unique role of morphometrics in interdisciplinary analyses.

The value of morphometric analysis for determining stock structure relies on appropriate methodology. Well designed sampling and analysis supports representativeness and reliability of results. As a distinct application of morphometric analysis, case studies in morphological stock identification offer methodological advancements and refinements that could be considered in other morphometric applications.

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