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USING MORPHOMETRIC AND MERISTIC CHARACTERS FOR IDENTIFYING STOCKS OF FISH

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INTRODUCTION

An animal species may be defined as groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups (Mayr 1963). Genetic or phenetic homogeneity among these groups over the entire distribution of the species is rarely observed due to heterogeneity and discontinuities in the environment or, simply, due to isolation by distance. Fish species are no exception to this generality and are usually subdivided into more or less distinctive groups which, in the case of commercially important species, are commonly referred to as stocks. I define stock as a self-sustaining group of individuals sharing a common and unrestricted gene pool. In terms of population genetics, a stock is a panmictic subunit of a species that is generally in Hardy-Weinberg equilibrium. Although random factors may influence geographic variability within a species, we generally assume that stock variability is important to the species for continued successful reproduction and adaptation. Therefore fish biologists have long sought to define stocks of fish, to understand the spatial and temporal dynamics of stock differentiation, and to apply these data to conserving and managing the species (see Canadian Journal of Fisheries and Aquatic Science, Volume 38(12)).

Identification of stocks of fish has long been the province of morphologists. Large data sets have been, and continue to be, collected for a diverse array of commercially important fish (see Table 1). But since the 1960s, there has been a surge of technical advances in the field of molecular biology (Ayala 1976; Nei and Koehn 1983) and the use of molecular characters in fisheries biology has increased dramatically (Ryman and Utter 1987). Theoretically, molecular data--from DNA to proteins--are superior for stock identification because of their direct and simple genetic basis. This accounts for the fact that three of the four papers in this symposium discussing character sets for identifying stocks of fish are based on molecular data. Still, in this flurry of molecular work, there have been parallel advances in the concepts and techniques of viewing, collecting, and analyzing morphological data. We may see a resurgence of morphological studies (pers. comm., J. Felsenstein, University of

Washington, Seattle, WA). Given the large number of molecular studies of fish, the best recourse for the morphologist is to continue to study morphological variability but to view it in light of its relationship with other molecular character sets. A better understanding of morphological characters will inevitably result. As Lewontin (1984) observed, "It often happens that the observed morphological differentiation is clear and statistically significant, while the differences in gene frequency are less powerful in discriminating populations and species."

My objective is to present some of the newest developments in the collection and use of morphological data for the identification of stocks of fish. I will focus on three areas: 1) types of characters, 2) data collection procedures, and 3) statistical analyses. Illustrations of these areas will be provided using data from my studies of milkfish (Chanos chanos) and chinook salmon (Oncorhynchus tshawytscha). I will also discuss several ways in which morphological data may be applied in concert with electrophoretic characters in management of fish stocks. This work is restricted to morphometric and meristic characters.

TYPES OF MORPHOLOGICAL CHARACTERS

A biologist studying morphological variation will make counts of elements in or along specific body parts and measure distances between distinguishing landmarks. In other words, he/she may collect meristic data and morphometric data. Standardized techniques for investigating these characters are described in Hubbs and Lagler (1947).

The most frequently used meristic characters are scales and fin rays. Scale counts described by Hubbs and Lagler (1947) include lateral-line scales, scales above and scales below the lateral line, circumferential scale count at the caudal peduncle and anterior of the dorsal fin, cheek scales, and scales before the dorsal fin. Counts of fin rays are taken for the median and paired fins. Other characters that are frequently used are counts of vertebrae, branchiostegal rays, pyloric caeca, gill rakers, and teeth.

For over 30 years, most morphometric investigations have based the selection of characters on the set of measurements described by Hubbs and Lagler (1947). These traditional morphometric characters measure length, depth, and width of fish shape, primarily in the head and tail regions. For example, in my study of milkfish, I collected data for the 16 characters depicted in Figure 1. These characters are not unlike those used in every study of morphometric variability listed in Table 1 (e.g., see Figure 1, Meng and Stocker 1984).

Recently, the use of these traditional morphometric characters has been questioned (Humphries et al. 1981; Strauss and Bookstein 1982; Bookstein et al. 1985). These workers argue that the conventional-type characters concentrate along the anterior-posterior body axis and in the head and caudal region and therefore produce uneven and biased areal coverage of the entire body form (see Strauss and Bookstein (1982) for details). Localized changes in body shape may go undetected, they argue, amidst the long distance measures across regions of the fish body. Their suggestion is to cover the shape or outline of a fish uniformly with a network of distance measures. This crisscross pattern along the body form is called a truss network (Humphries et al. 1981). Theoretically, this systematic characterization of the geometry of a fish form will increase the likelihood of extracting morphometric differences with biological meaning within and between species.

To establish a truss network pattern, morphological landmarks are identified along the outline (or surface) of a fish. Good landmarks are not identified by extremities, like the narrowest portion of the caudal peduncle, but by anatomical features. "Anatomical landmarks are true homologous points identified by some consistent feature of the local morphology" (Strauss and Bookstein 1982). For example, I used the 12 landmarks illustrated in Figure 2. There are numerous uses of conventional landmarks in a truss network (e.g., the origin and insertion of fins). I also found good results with points like #1 (Figure 2), the posteriormost point of the maxillary, at the closest point to the body on a line perpendicular to the horizontal axis of the specimen; point #4, posterior aspect of the neurocranium; and points #11 and #12, anterior attachment of the membrane from the caudal fin. Ideally, an equal number of dorsal and ventral landmarks are used. Six distance measures connect a set of two dorsal and two ventral landmarks, producing four peripheral distances and two diagonals. A set of six measurements for four landmarks is considered a cell. Five cells have been constructed across the form in Figure 2. In this case, with 12 landmarks, five cells are constructed yielding 26 distance measurements. Cells and truss characters may be referenced according to the scheme of Strauss and Bookstein (1982). For example, the distance between landmarks 1 and 2 is truss character 1-2 in cell 1, the distance between landmarks 9 and 11 is 9-11 in cell 5, and so on.

Will truss characters help in resolving stock differences? Humphries et al. (1981) and Strauss and Bookstein (1982) present several examples illustrating how truss network characters are better for discriminating between two species than are conventional characters. I have seen similar results working with chinook salmon. Namely, I collected juvenile chinook salmon in three locations and took 11 conventional and 33 truss measurements

on each individual (Figure 3). The results of a discriminant function analysis of the two data sets are presented in Figure 4. A scatterplot of scores from the analysis of conventional measures indicated minor between-sample differences (Figure 4A). In contrast, the analysis of the truss characters indicated there was essentially complete discrimination among the three samples (Figure 4B). The details of a similar comparison of groups of Pacific salmon have been reported (Winans 1984).

An apparent drawback to truss characters is making the larger number of measurements crisscrossing over the fish form--a caliper nightmare. New developments in electronic devices discussed below, however, have solved this problem.

COLLECTION OF DATA

> "If you want to count scales or gill rakers... go ahead." (Anon.)
>
> "Dividers or a dial-reading caliper should be used for measurements." (Hubbs and Lagler 1947)

Many meristic counts can be made by eye, depending on the specimen size and the character. In some cases for certain scale or ray counts, dissecting microscopes are necessary. In other cases, characters like vertebrae are counted from negatives of x-ray photographs. Frequently, a red dye may help identify special characters, like mandibular pores in salmonids (pers. comm., R. Leary, U. Montana, Missoula, MT). I am aware of one attempt to automate the collection of meristic data. McAllister and Planck (1981) describe an automatic counting probe, which can be attached to a computer or data-recording device. Their description:

> "The automatic counter consists of a pen-like touch-sensitive probe, whose spring-loaded tip adds one to the count each time it is lightly pressed against a series of images or objects. Countable items might include fish scales, vertebrae on radiographs, or fishes in photos of schools. When the last item is counted, the SEND button at the top of the probe is pressed, transmitting the count to the computer and resetting the display counter to zero ready for the next count."

My reservations, without actually trying out the device, concern the mechanical sensitivity and facility of using the device on small elements such as gill rakers viewed under a microscope. Experimenting with this type of data-collection device may eventually lead to a versatile and time saving tool.

The collection of morphometric measurements can still involve hand-held calipers, meter sticks, and/or a measuring board. Once the data are taken in this fashion, they are generally keypunched into a computer for analysis. Several new electronic devices with computer orientation are available which speed up the process and eliminate some of the errors involved in collecting morphometric data. I will discuss in detail the use of a digitizing board because of my experience with it, and will briefly mention several other new devices.

Distances between morphological features on a fish can quickly and precisely be determined in the laboratory with an X-Y coordinate digitizing pad. The researcher first records the positions of certain morphological features or landmarks around the outline of a specimen using a set of X-Y axes on a digitizing pad. Distances between landmarks are then calculated from the X-Y data. I have described a 3-step process (Winans 1984) for collecting distance measures in this fashion. The following is a summary of the procedure.

1. Positioning

   Specimens are placed on water-resistant paper, and body posture and fin positions are teased into a natural position. Positioning of specimens in this fashion is a precise process, as evidenced by low measurement error (see Winans 1984, Table 1).

2. Pinning

   Distinctive and homologous landmarks are selected around the outline of the fish form. Each landmark is indicated and recorded by making a hole with a dissecting needle in the water resistant paper alongside its respective location. Data such as specimen number, body weight, and color are recorded alongside each specimen and added to the computer file when the landmark holes for that specimen are digitized.

3. Digitizing

   After the landmark information from a set of specimens has been recorded (pinned), the paper is placed on an X-Y coordinate digitizing pad to establish a reference set of X and Y axes to view interlandmark distances. The X-Y coordinate values ($\pm$0.01 mm) for positions of the landmarks are indicated and recorded on a computer by depressing an attached digitizing stylus into each hole. Landmarks are digitized in the same sequence for each fish. Specimen identifying data are added via a computer terminal/keyboard and also stored with the digitizing information. The Euclidean or

morphometric distances between pairs of landmarks are then calculated by computer (using the Pythagorean theorem.)

This digitizing procedure speeds up and adds flexibility to the process of collecting morphometric data. As an example, I pin 80-100 specimens in an 8-hour day and later can digitize and calculate morphometric distances for 100 fish in about 90 minutes. This is much faster than measuring specimens with hand-held calipers. And importantly, the faster data-collection process facilitates collecting synoptic data (e.g., morphological and electrophoretic data) from the same specimens.

The use of a digitizing pad leads to procedural and analytical flexibility. Recording X-Y data for relevant landmarks on a fish outline provides the morphometrician with the capability of selecting traits for analysis without the need to remeasure specimens. For example, both a truss network and a conventional data set can be calculated from the same set of digitized landmarks and compared. Since the landmarks are essentially in a 2-dimensional plane, statistical adjustments for folded or twisted fish are not necessary (see unfolding statistics in Strauss and Bookstein 1982). Also, because of the 2-dimensional nature of this setup, digitizing fish shapes from photographs is possible with a high degree of precision (unpublished data).

Several other electronic devices should be noted. McAllister and Planck (1981) describe automatic calipers which are designed to transmit measurements to a computer, pocket calculator, or data-recording device. Although the selected calipers will limit the size of fish that can be measured, the procedure is accurate and fast. With a battery power supply, the setup becomes portable for field work.

Recent advances have been made in image processing that might also be applicable to the study of body outlines of fish (Ferson et al. 1985 and references therein). Basically, the procedure involves placing an object on a screen and tracing its silhouette by digitizing. The resultant closed curve is analyzed by fitting a set of mathematical functions (e.g., elliptic Fourier approximations). The Fourier coefficients are then analyzed with conventional multivariate statistics to view between-group differences. Notably, this image processing is done without homologous landmarks. Could it work with fish? Ferson et al. (1985) write,

> "Because the present elliptic Fourier methods do not need continuous traces for input and work for any sequence of two-dimensional points, when landmark data are endowed with a natural or arbitrary order, elliptic Fourier description should be adequate to capture variation in relative landmark positions."

This procedure is currently being tested on fish (pers. comm., S. Ferson, State Univ. New York, Stony Brook, NY). Some authors question the usefulness and biological meaning of describing closed shapes with Fourier descriptors (Bookstein et al. 1982). As Ehrlich et al. (1983) point out, there is need for more empirical studies where the efficacy and interpretability of one technique can be compared with another. Image processing may prove to be a powerful technique for describing shapes in some instances.

STATISTICAL ANALYSES

There is a basic difference between meristic and morphometric characters. Meristic characters are discrete, and can assume only integer values. In contrast, morphometric characters are continuous and assume the values of real numbers. Therefore, meristic and morphometric characters should not be considered in the same statistical analysis (Seal 1964). Moreover, the data should be transformed differently. It is frequently observed that measurement means and variances are correlated, the largest characters like fork length or lateral-line scales having the largest associated variances within their respective data sets. To decrease the effect of this correlation, the raw data are transformed. Sokal and Rohlf (1981) recommend transforming meristic characters to square roots and a $\log_{10}$-transformation of morphometric data. The latter transformation preserves allometric relationships among the characters (Jolicoeur 1963). For a multivariate analysis, such as principal component analysis, an alternative solution for merisitic characters is to use a correlation matrix instead of a variance-covariance matrix.

With the collection of large sets of morphological data, how should the data be analyzed: univariately, bivariately, or multivariately? The answer depends on how we view morphological adaptation and evolution. I concur with Sokal and Rinkel's (1963) multivariate perspective:

> "Geographic variation is not likely to be due to adaptation of a few characters to a single environmental variable, but is doubtless a multidimensional process involving the adaptation of many characters to a variety of interdependent environmental factors...".

Thus, a correct understanding of morphological variation is multivariate (Gould and Johnston 1972). We ought to strive to examine thoroughly the patterns of variance and covariance among all characters in a data set using multivariate statistics.

Multivariate analyses of morphometric data sets usually identify size and shape differences among individuals and groups. In compliance with current morphometric work, size and shape are considered factors--linear combinations of variables. Size is defined here not as a single character, but a factor that can predict any distance measurement (Humphries et al. 1981). Shape is defined as a specific relationship among characters as described by specific correlations, +, -, or 0, between the characters--a measure of geometry. For most stock identification work, shape discriminators are desired, as we can usually sort fish to size quite readily just by eye. Unfortunately, shape measures are not independent of size because of allometric relationships, and size-free shape estimators are difficult to obtain. This problem is discussed in detail in Humphries et al. (1981). I will present an overview of their arguments and recommendations.

There are three general approaches for removal of size influences in analyses of shape: ratios, regressions, and multivariate analysis. Simply stated, it is believed that the division of a character by a measure of size, say, fork length, will produce a size-free measure of that character. Similarly, if a measure is regressed against say, fork length, replacing the original measurement by its residual after regression will produce a size-free measurement (Thorpe 1976). The principal argument of Humphries et al. (1981) is that these ratios or regressions only remove the effect of the one variable, e.g., fork length, from the measurement. The third possibility for producing size-free shape components is through multivariate analyses such as discriminant function and principal component analyses. Humphries et al (1981) reject discriminant function analysis as a descriptive tool because of the difficulty in interpreting the coefficients in a biological context. For example, the interpretation of shape components is based on the coefficients in the discriminant function vectors. However, as Humphries et al. (1981) point out:

> "From within a set of correlated characters only the variable with the highest F-statistic will be weighted heavily. Within that set, variables that do not contribute added discrimnation will have low coefficients even though they contain nearly as much information about shape as the variable with the high F-statistic."

Campbell and Atchley (1981) and Williams (1983) likewise question the interpretability and stability of discriminant function coefficients. The recommendation of Humphries et al. (1981) is to use principal component analysis to view multivariable data sets. In principal component analysis individuals are not assigned a priori to groups, thus permitting "group differences to be

discovered." Moreover, principal component coefficients are essentially the covariance of the measurement on the component axis, and are thus amenable to biological interpretation. Before describing their new approach to making size-free shape components, I will briefly describe principal component analysis.

Principal component analysis computes a set of uncorrelated composite variables called principal components (hereafter PCs) from a variance-covariance (or correlation) matrix (Dunn and Everitt 1982). The first principal component (referred to as PC I) explains the most variance in the data set. Geometrically, PC I is thought to lie parallel with the largest axis in the hyperdimensional cloud of data (see Campbell and Atchley 1981; Green 1976). PC II is independent of PC I, that is, it lies perpendicular to the axis of PC I, and explains the second largest component of variation in the data set. PC III is independent of the other PCs and explains the third most variation, and so on for the other PCs. Each PC is a linear combination of the variables and is defined by a vector (an eigenvector) of coefficients and an eigenvalue. The coefficients are essentially a measure of covariance of the character on that PC. The eigenvalue is a measure of variability explained by a particular PC; the sum of the eigenvalues equals the total variability in a data set. Since on any component only a few characters have large coefficients, the biological interpretation of a component is based on the magnitude and signs of these so-called important characters. Examples of this are given below.

What about size-related problems? PC I characteristically has + signed coefficients for all measurements and is interpreted as a size vector. Samples and individuals sort by overall size on PC I. Subsequent components describe specific covariability relations or shape, as variables have + or - signed coefficients or are zero. Frequently, though, residual size effects are observed in these shape components. For example, in a plot of PC scores for a particular component, say, PC II onto the PC I axis, the ellipse of points for a sample is at a diagonal to the PC I axis instead of parallel to it. In other words, values of PC II are not independent of the size axis, PC I. Humphries et al. (1981) describe and illustrate a multivariable method called shear analysis for removing size from PC scores and vectors. It is a modified principal component analysis and uses scores from a second principal component analysis of centered (mean-adjusted) data by group to remove size influences (see p. 300, Humphries et al. 1981, for the six steps in shearing data; or Bookstein et al. 1985).

To illustrate visually how shearing works, I will use results from an analysis of morphometric data from chinook salmon. Judging from the eigenvectors (data not presented here), PC I was a size-related axis and PC II was a shape axis. A plot of scores

on PC I and PC II is presented in Figure 5A. Clearly the scatter of points for each of the two samples is oriented at a diagonal with PC I. Namely, the larger the fish, the smaller the PC II value. Following a shear analysis, the orientation of the scatter of points in each group is parallel with PC I (Figure 5B). Shape variability along the PC II axis is now independent of size. Any component other than PC I, whether from a merisitic or morphometric data set, can be sheared in this way to eliminate size effects.

Presumably, we have arrived at a set of techniques which view character variance and covariance in large data sets to produce multivariate size and size-free shape descriptors. In conjunction with shear snalysis, principal component analysis provides a set of rules, defined by shape eigenvectors, that define new shape variables. Scores on these shape components can then be evaluated for significant between-group differences in routine analyses, such as analysis of variance or multiple range tests.

MODEL FOR STUDYING TEMPORAL STABILITY

One of the principal problems in the use of morphological characters for stock identification is that morphological phenotypes are labile to environmental variability (discussed below). Therefore, before implementing size-free shape components in stock identification programs, we must examine the temporal stability of the multivariate relationships. It is important to know if between-year variability is less than between-stock variability for a given shape discriminator. I have outlined in Figure 6 a simple model for examining temporal stability in these characters. It requires a minimum of two years of data collection. It is applicable to either meristic or morphometric data, although in the figure and text I refer to the set of important characters in a component as a shape descriptor. For two years of data, there are two steps: Step 1 is a search for a size-free combination of morphological variables that is a good stock discriminator, and Step 2 is to determine the temporal stability of the discriminator and the respective differences among locales.

In the first year, data are collected from specimens from various locales, preferably while the fish are segregated onto spawning locations. A principal component analysis produces a size factor, PC I. Subsequent components are sheared (when necessary) to produce size-free components. Analyses are conducted on the scores from the sheared components to test if significant between-locale (stock) differences exist in the data and in what pattern.

Step two is essentially a repeat of Step one, except that the researcher tests the temporal nature of the results first revealed in year 1. Namely, he/she can first examine the correlation of eigenvectors, i.e., the correlation of the coefficients of sheared PC II from years 1 and 2; and secondly, examine the pattern of mean values by locale from Year 1 and 2. Nonsignificant differences between years for both of these tests add a great deal of confidence in the use of the respective PCs as stock discriminators. As illustrated in the bottom portion of Figure 6, the data from the two years may be pooled, and the principal component scores on the sheared axis (or axes) examined in a 2-way analysis of variance. In this analysis, the researcher can quantify differences in shape due to geography (between stocks) and to time (between years within locales). The most useful results with respect to stock identification are when stock shape values do not vary significantly from year to year. This does not mean that the morphological variability is primarily genetically determined, only that the influence of yearly environmental changes is less than the geographic differences. Note that this model is applied separately for meristic and morphometric characters. I will demonstrate the use of the model in the following example.

Example 1. Morphometric variation in juvenile chinook salmon.

I am interested in evaluating morphometric variability among stocks of juvenile chinook salmon for use in identifying the origin of fish while in mixtures in an estuarine or nearshore marine environment. In 1982 and 1983 fish were collected in estuaries and rivers along the Oregon coast (Figure 7A). They were frozen and taken to the laboratory for electrophoretic and morphometric evaluation. For simplicity, I report here the results of analyses on the four most geographically separated samples: Nehalem, Tillamook, Coquille, and Sixes.

Descriptive statistics of the samples are given in Table 2. Twenty-six truss network measurements were made on each fish using 20 digitized landmarks. The first principal component, PC I, explained 88% of the total variance and was a size-related component. Coefficients were roughly equal and positively signed for all variables on this component (Table 3). PC II and III explained 3 and 2% of the total variance, respectively. Other components explained less than 1% of the variance and are not considered further. The second and third components were sheared, producing the size-free shape components, sheared PC II (SPC II) and sheared PC III (SPC III). Important characters in both of these components were located in the tail, involving landmarks 9-12. An analysis of variance of SPC II and III scores indicated significant between-locale differences. Results of a Duncan's multiple range test of PC scores are displayed in Table 4. Fish

from Location #4 in the south (Sixes River) were significantly different from the other three locations on both SPC II and SPC III. In accord with Step 1 of the model (Figure 6), tail shape differences exist among four samples collected in 1982.

The 1983 samples, described in Table 2, were analyzed in the same fashion. Again, PC I was a size-related component and explained 89% of the total variance. Components II and III each explained 2% of the variation; they were sheared to produce size-free components. The correspondence between the eigenvectors of 1982 and 1983 was high, for example, characters 9-11 and 10-12 had the largest coefficients for SPC II and SPC III, respectively, in both years. The correlation of coefficients for 1982 and 1983 was 0.86 for SPC II and 0.76 for SPC III. However, the correspondence of the sample means on these two components between 1982 and 1983 was low, as shown in Table 4. In fact, the pattern of geographic variation was reversed from that seen in 1982. Although the three northern samples were still not significantly different at SPC II, the Sixes River sample now had the highest value of SPC II in 1983.

The 1982 and 1983 data were pooled for a principal component analysis to assess further geographic variability in light of annual variability. The eigenvectors were similar to those from the independent analyses (Table 3). The results of a multiple range test in Table 5 highlight the heterogeneous nature of the results seen in Table 4. For instance, Sample #3 (Coquille River) had the largest SPC II value in 1982 and the smallest in 1983. The results of a 2-way analysis of variance confirmed these findings. For scores on SPC II and III, the amount of between-year variance, as measured by F-values, was from 3 to 30 times greater than the between-locale variance. Clearly there is no temporal stability to the pattern of geographic variation in these samples.

A consideration of some preliminary growth studies of chinook salmon aids the interpretation of these results. The early life history of Pacific salmon is marked by a smoltification period during which considerable physiological, biochemical, and behavioral changes occur as the young fish prepare for the transition from freshwater to seawater (Folmar and Dickhoff 1980). I have studied body shape changes during early development in chinook salmon reared in hatcheries and reported a dramatic change in the shape of the caudal peduncle presumably associated with smoltification (Winans 1984). The pattern of change seen in a sample of hatchery fish along the SPC II axis is illustrated in Figure 8A. The important characters associated with these changes are illustrated in Figure 9A. Interestingly enough, the same characters are contrasted in the SPC II component in the above

study of wild chinook salmon (Figure 9B). For a comparison, mean SPC II scores for the eight Oregon samples are plotted in Figure 8B. A similar pattern of shape change with growth is seen. Apparently, discrimination among these samples is more a function of the degree of smoltification than true geographic differentiation.

With respect to the model in Figure 6, my conclusions are that in Step 1, tail shape characters were identified and inter-locality differences noted, and in Step 2, tail shape variability was again detected, but yearly variability was greater than between-locality differences. Other studies indicate that shape differences are related to ontogenetic differences.

I present the following study to demonstrate the use of a principal component analysis to describe a simple rule for identifying fish to group.

Example 2: Meristic and morphometric variability in milkfish.

I have investigated morphological and electrophoretic variability in milkfish from 15 locations in the Pacific Ocean (Figure 7B). One of the major observations is that fish from the Philippines differed electrophoretically and morphometrically from neighboring samples along the equatorial Pacific Ocean (Winans 1980; Winans 1985). In this example, I use the two southern Philippine samples P1 and P2 and the nearest sample from the equatorial Pacific island group, Palau (PAL), to demonstrate a particular use of principal component analysis.

I examined 6 meristic characters and 19 traditional morphometric characters on each fish. A principal component analysis of the meristic data transformed to square roots indicated extensive overlap among the samples and was not considered suitable for stock identification (see Figure 5, Winans 1985). A principal component analysis of log-transformed morphometric data revealed size differences along PC I, and considerable variation along the sheared PC II axis. There was a basic dichotomy in SPC II scores, viz., Philippine samples, differed from the non-Philippine samples, with one Philippine sample, Tahiti, and Christmas Island samples adding heterogeneity to this general pattern (Figure 10). Although a shape change associated with size was apparent in Hawaii, the magnitude of this change was not greater than, or overlapped with, the SPC II dichotomy discussed here. The difference between the Palau sample and the two samples from the Philippines is illustrated in a histogram of the SPC II scores (Figure 11A). The two groups do not overlap on this character axis.

Six characters had relatively large coefficients for SPC II (Table 6). Several head characters had large, positively signed

coefficients (orbital, snout, and premaxilla lengths), contrasted with three tail characters with negatively signed coefficients (caudal depth, body depth at anus, and length of anal fin base). My biological interpretation of this shape component based on the eigenvector is that the Philippine samples have smaller heads and larger tails in comparison to the Palau sample. For practical reasons, I wanted to see if I could go one step further than just identifying the important characters on a vector. I wanted to know whether this smaller set of important measurements by itself could be used to discriminate these samples. To test this, new SPC II scores were calculated for each fish using data from only these six variables. As an example of the calculations, the calculation of a SPC II score for fish $\underline{i}$ is:

$$\text{SPC II}_i = \text{snout length}_i\ (0.317) + \text{orbital length}_i\ (0.397)$$

$$+ \text{caudal depth}_i\ (-0.350) + \text{body depth}_i\ (-0.515)$$

$$+ \text{anal fin base}_i\ (-0.253) + \text{premaxillary length}_i\ (0.258).$$

(Note that these values will differ from Winans (1985) because overall character means were not subtracted first from each variable, i.e., absolute values differ but the relative values do not.) The SPC II scores, calculated from these six variables are plotted in Figure 11B. The difference between the two Philippine samples and Palau sample did not decrease, but in fact, increased slightly. I conclude that a principal component analysis of 19 morphometric characters identifies shape differences associated with the head and tail regions of the fish. There is no loss of discriminatory power when only the six most important characters are used to calculate a SPC II score.

DISCUSSION

One of the most important recent developments in evaluating morphometric variability in fish is the truss network character set. It clearly is an objective procedure for uniformly covering the outline of a fish with distance measures for shape analysis. First, albeit few, applications of this technique indicate truss characterization of shape is more sensitive for detecting differences among species and, as is relevant here, among stocks. It could be argued that enhanced discrimination with truss data is due simply to the increase in absolute number of characters presented for analyzing. Whereas $\underline{n}$ characters will generally provide better discrimination than $\underline{n}$-1 characters (Speilman and Smouse 1976), I think in this case shape discrimination also increases due to the addition of more information about local changes in body shape. The generality of this technique will be tested as more traditional and truss character sets are compared.

Considerable advances have been made recently in the development of electronic equipment suitable for morphological investigations of fish. My focus here has been on digitizing the X-Y coordinates of morphological landmarks using an X-Y digitizing board. The digitizing procedure is fast, produces data sets amenable to the calculation of various types of distance measures, and it is precise. I routinely collect morphometric and electrophoretic data from the same individuals. I can quickly thaw specimens (frozen to preserve proteins), identify the relevant landmark positions by pinning, and then refreeze the specimens at a rate of 80-100 specimens per 8 hours. Landmark information for the 80-100 fish can then be digitized in about 90 minutes. Importantly, because of the fast pinning process, tissue preparations from the refrozen specimens can later be subjected to electrophoresis without any detectable change or deterioration in electrophoretic banding patterns. Moreover, measurement error in this process is small, less than 0.5 mm for most measurements (Winans 1984). A greater use of digitizing equipment in this area of research will eventually lead to an increase in the quality and quantity of information that can be gleaned from fish shapes.

Other technical developments also look promising. Technical advances in the field of image analysis will be followed closely by morphometricians (Rohlf and Ferson 1983). When a structure or outline is free of landmarks, e.g., bones or otoliths, perhaps the best approach will be to apply image analysis in conjunction with Fourier descriptors. But further investigations into the application of Fourier analysis of closed shapes are encouraged to resolve any questions and problems associated with this potentially powerful technique (see Bookstein et al. 1982; Ehrlich et al. 1983). Developments in the field of ultrasound digitizing, including 3-dimensional viewers, are also being examined as tools for fish morphometrics (pers. comm., A. Johnson, NMFS, Panama City, Florida). As a greater variety of techniques for collecting and viewing morphological data become available, I foresee a need for more comparative studies to determine which techniques will yield the best, most reliable discrimination. As Ehrlich et al. (1983) state: "There is no reason to expect that one morphological technique will yield equally good information for all investigators," or for all species.

Principal component analysis was presented here as a useful multivariate statistical procedure for viewing multicharacter variability within and among groups of fish. Principal components describe the major axes of character variability in simple character space; typically the first few components contain most of the variability in the data set. Clearly, understanding variation at a few composite PC variates is easier than trying to understand greater then n patterns of variability at n variables. The relative contribution of a variable to a PC variate is determined by the relative size of its PC coefficient. Thus, PC

analysis can be used to identify the important variables. If a large number of variables are examined in a pilot project, the number of variables which must be measured or counted in a subsequent, large scale study may be reduced. This was demonstrated in a simple case here for milkfish (Example 2). By interpreting the PC results in Table 6 as I did, I eliminated about 2/3 of the morphometric characters without a loss of stock discrimination (Figure 11).

Determining the number of PC components for analysis can be subjective. Frequently, components are dismissed if their associated eigenvalues are less than 1.0 (Tatsuoka 1971). In Example 1, I dismissed components after PC III, because the amount of variation explained in each of these components was 1% or less. To reduce this source of subjectivity, Gibson et al. (1984) have applied the jackknife procedure to principal component analysis. This technique provides estimates of variance of the coefficients in the eigenvectors and of the eigenvalues. This is done by iteratively removing data for one individual and redoing the component analysis. They demonstrate how it is used to identify stable, interpretable coefficients, and feel that this approach "should restrain a general tendency to over-interpret." The jackknife procedure, as a method to improve the statistical robustness of principal component analysis in stock identification, should be explored further.

The primary frustration in the use of morphological variation for stock identification is that the variability is not simply or directly inherited. It is generally assumed that substantial, but usually unknown, amounts of environmental influence may be involved with patterns of morphological variability. Some meristic characters are quite heritable (e.g., Leary et al. 1985a reviewed in Kirpichnikov 1981), but we know almost nothing of the genetic basis for the multivariate meristic or morhometric characters as defined by principal component analysis. The genetic basis of, say, head length for milkfish is unknown at the present time, not to mention the genetic basis for the composite variable SPC II discussed here. It is encouraging that recent work with rodents has shown that multivariate shape characters defined by multivariate analyses have a substantial heritable component (Atchley et al. 1981; Leamy and Atchley 1984; and Leamy and Thorpe 1984). Obtaining comparable estimates for most fishes is technically unrealistic now. It is more practical to evaluate the temporal stability of multivariate morphological components to determine their reliability as practical stock descriptors.

A model is presented for examining temporal stability of morphological variation among smaples (Figure 6). It is an intuitively simple program for analyzing morphological data that has been collected for a <u>minimum</u> of two years. The model is based

on a multivariate analysis to describe multicharacter complexes as defined by specific eigenvectors. Each fish has a single value on each component. Therefore, it is the eigenvectors and their respective component scores which require evaluation. Suggested analyses are correlation studies of the eigenvectors and analysis of variance of the component scores. Although a few workers have collected two years of data in a study, none have completed any of the informative analyses presented here (e.g., Todd et al. 1981; Riddell et al. 1981). Studying quantitative characters (like fish shape) "is a difficult and somewhat slippery affair" (Lewontin 1974). Sound sampling and statistical analyses as suggested here will give satisfactory evaluation of morphological characters. Further understanding of the forces that direct morphological characters, be they environmental or genetic, may be gained in multicharacter studies of changes in the environment and genetic structure.

Since the advent of various molecular techniques in the 1960s, considerable expertise and extensive data bases have developed with respect to population genetics of fish. Provided with readily available genotypes, fish biologists interested in morphological variation can gain new and valuable perspectives of morphological variability. Information of the association of morphological phenotypes and biochemical genotypes may be useful in fish management and conservation programs (see Soule 1980). I present two examples, one at the individual level and one at the stock level, illustrating the complementary use of morphological and molecular (in this case electrophoretic) characters.

Asymmetry of bilateral meristic characters. Bilateral characters in organisms are usually not perfectly symmetrical. The number of rays in the left pectoral fin of a fish may not equal the number of rays in the right pectoral fin. Numerous studies of fish have looked at levels of asymmetry (e.g., Felley 1980; Graham and Felley 1985; Angus 1982). The findings of Leary et al. (1984) are perhaps most pertinent to the management of fish stocks. Leary and his colleagues have examined electrophoretic variability (measured as heterozygosity) and asymmetry in several salmonids. Their general conclusion is that there is a negative relationship between heterozygosity and asymmetry between individuals within a population. That is, individuals with the most heterozygous loci are likely to have the fewest number of asymmetrical characters. Moreover, they have noted that obviously deformed rainbow and cutthroat trout are more asymmetrical in their bilateral characters than are normal individuals (see Figure 1, Leary et al. 1984). They conclude that asymmetry may be negatively correlated with biological fitness. Concerning measurements of asymmetry of meristic characters, they write (Leary et al. 1985b):

> "We envision the most valuable use of this technique to be the monitoring of populations through time. A progressive increase in average asymmetry would indicate a loss of genetic variation through inbreeding or an increase in environmental stress. The ideal monitoring program would combine an examination of allele frequency changes at isozyme loci and changes in fluctuating asymmetry. Such a program would be able to both detect the loss of genetic variation and simultaneously evaluate the effects of such loss on the population."

As a potential tool for use in fisheries, as Leary et al. envision, it is important to determine the relationship, if any, between bilateral meristic asymmetry and electrophoretic variation in other commercially important fish.

Characters used in mixed stock fisheries. A common practice in fisheries science is to estimate proportions of various stocks in a mixed-stock fishery, when samples and baseline data are available from all contributing stocks (Milner et al. 1985). An important issue in problems of mixed stock fisheries is the selection of characters. Ideally characters are discrete, expressed independently of environmental variation, temporally stable, and cost effective. Allele frequency differences at protein-coding loci detected by protein gel electrophoresis generally fulfill these requirements (e.g., Grant et al. 1980; Beacham et al. 1985a and 1985b).

The work of Fournier and his colleagues has expanded the statistical model for mixed stock fisheries in two important ways. First, they have extended the model to include the use of several types of continuous and/or discrete characters simultaneously (Fournier et al. 1984). Conceivably the model can accept principal component scores of morphological data that are shown to be temporally stable. Furthermore, Fournier and his colleagues are working on another version of the model which may also accommodate so-called nonstationary characters (pers. comm., C. Woods, Fisheries and Oceans, Nanaimo, Canada). Nonstationary characters are characters that vary from year to year, and in one year, may or may not be helpful discriminators, and/or are characters which can not be measured for a database, and their stock specificity is unknown. In the proposed mixed fisheries model, each iterative step of the maximum likelihood analysis makes estimates of the stock proportions and the proportions of the nonstationary characters in the contributing stocks. The latter estimates are then reapplied to the next stock estimates. If nonstationary characters vary sufficiently among stocks, their inclusion will help stock estimates, otherwise, nonstationary characters will not affect the process. This means then that

characters such as meristics and morphometrics, as well as scale patterns, parasites and egg size will only positively affect stock estimates. The concept of this model epitomizes the use of multicharacter data for solving a fisheries problem.

In summary, I have presented some of the recent developments associated with collecting and applying morphological data in identifying and managing stocks of fish. It has been observed that many of the disciplines in biology that once were the exclusive domain of morphology have been assumed and, in some instances, taken over by molecular-oriented technology (e.g., Lewin 1985). Therefore, I have concluded this paper by presenting examples of how combinations of morphological data and molecular data (i.e., electrophoretic) can be potentially more useful than either character set alone in both genetic conservation programs and management programs. We have a lot to learn about fish genotypes and phenotypes; examining the association of different character sets at the individual and population levels is an important first step in this field of research. I feel we should continue our research of morphological variability in fish, especially in coordination with research of other character sets, testing and using as many new ideas and technologies as seems necessary and appropriate.

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Table 1. Representative morphological studies of stock structure in commercial fishes.

FRESHWATER	
Bluegill	Felley 1980
Brook trout	McGlade and MacCrimmon 1979
Lake whitefish	Casselman et al. 1981 Ihssen et al. 1981
DIADROMOUS	
Atlantic salmon	Riddell et al. 1981
Pacific salmon	Beacham 1985 Hjort and Schreck 1982 Winans 1984
Smelt	Copeman 1977
Shad (meristics only)	Gabriel et al. 1976
MARINE	
Herring	Meng and Stocker 1984
Milkfish	Winans 1985
Capelin	Sharp et al. 1978
Flounder	Wilk et al. 1980

Table 2. Descriptive statistics of juvenile chinook salmon collected in August-September of 1982 and 1983. Sample locations illustrated in Figure 7.

		1982				1983			
		Sample size	Fork length (mm) minimum	mean	maximum	Sample size	Fork length (mm) minimum	mean	maximum
1.	Nehalem	42	109	120	143	11	127	138	152
2.	Tillamook	56	97	121	147	26	104	127	165
3.	Coquille	51	92	110	132	24	98	106	122
4.	Sixes	50	82	96	112	25	92	114	126

Table 3. Variable coefficients on principal components I through III. Components II and III were sheared by method in Humphries et al. (1981) to produce size independent components. Coefficients are X 100. Refer to Figure 2 for characters.

	PC I			Sheared PC II			Sheared PC III		
Character	1982	1983	Pooled 82 & 83	1982	1983	Pooled 82 & 83	1982	1983	Pooled 82 & 83
1-2	14	15	15	-11	-5	-12	7	5	3
1-3	20	18	20	-9	-20	-7	-16	-5	-15
2-3	17	17	17	-9	-12	-10	-3	2	-4
1-4	16	15	16	-6	0	-6	-2	40	2
2-4	14	14	14	-8	-2	-7	-2	8	0
3-4	19	19	19	-5	-8	-10	0	3	4
3-5	20	21	20	-5	-13	0	-4	-14	-17
4-5	21	21	21	-6	-11	-4	-2	-8	-8
3-6	19	21	20	-7	-1	-4	-2	-14	-5
4-6	21	21	21	-4	-5	-2	-2	-22	-9
5-6	20	22	21	-11	-1	-12	-1	-1	2
5-7	21	20	20	-13	6	-2	-2	-1	-15
6-7	20	21	21	-10	-1	-6	-2	3	-7
5-8	20	22	21	-10	2	-11	-1	-6	3
6-8	18	19	17	-7	-16	-2	-4	43	-11
7-8	22	21	22	-10	4	-7	-2	-12	-3
7-9	19	17	19	-4	-20	-7	-6	1	-4
8-9	21	20	21	-3	-3	1	-6	-13	-10
7-10	20	20	21	-6	-5	-5	-13	-8	-8
8-10	24	21	22	3	-2	19	-35	-43	-41
9-10	19	21	20	-9	3	-16	20	20	22
9-11	24	23	23	80	85	87	-39	-9	0
10-11	19	19	19	18	23	8	22	27	34
9-12	21	21	21	30	11	23	11	13	18
10-12	18	17	19	32	7	5	74	56	70
11-12	19	19	19	-8	-20	-16	13	15	12
% of total variance explained	88	89	88	3	2	3	2	2	2

Table 4. Results of Duncan's Multiple Range Test of sheared PC scores of chinook salmon. Solid horizontal lines indicate samples which are not significantly different. Component scores were calculated from two independent principal component analyses of 1982 and 1983 data. From north to south, sample codes are 1 = Nehalem, 2 = Tillamook, 3 = Coquille, and 4 = Sixes. Mean component scores (X 10,000) are presented below sample codes.

	Sheared PC II				Sheared PC III			
1982	4 (-345)	1 (74)	2 (76)	3 (184)	1 (-163)	2 (-75)	3 (-6)	4 (300)
1983	3 (-334)	1 (-56)	2 (95)	4 (247)	3 (-167)	4 (-102)	1 (5)	2 (247)

Table 5. Results of Duncan's Multiple Range Test of sheared PC scores of chinook salmon. Solid horizontal lines indicate samples which are not significantly different. Component scores were calculated from a principal component analysis of the covariance matrix of pooled data from 1982 and 1983. PC scores were sheared by method of Humphries et al. (1981). From north to south, sample codes are 1 = Nehalem, 2 = Tillamook, 3 = Coquille, and 4 = Sixes; collection dates are 1982 (= 82) and 1983 (= 83).

1982 + 1983 data

Sheared PC II

3-83 4-82 1-83 2-83 4-83 2-82 1-82 3-82

Sheared PC III

1-82 2-82 3-82 4-82 3-83 1-83 4-83 2-83

Table 6. Variable coefficients on principal components I and II from an analysis of morphometric characters in milkfish. PC II was adjusted or sheared by algorithm in Humphries et al. (1981).

Morphometric character	PC I	Sheared PC II
Fork length	0.222	-0.131
Length snout-anal fin origin	0.222	-0.132
Length snout-pelvic fin origin	0.210	-0.101
Length snout-pectoral fin origin	0.212	0.177
Length snout-dorsal fin origin	0.223	-0.107
Head length	0.214	0.232
Snout length	0.249	0.317
Postorbital length	0.222	0.089
Orbital length	0.199	0.397
Caudal depth	0.215	-0.350
Body depth at anus	0.228	-0.515
Length dorsal fin base	0.223	-0.117
Length anal fin base	0.221	-0.253
Length pectoral fin base	0.252	0.025
Pectoral fin length	0.239	0.215
Head width	0.247	-0.095
Nares width	0.267	0.067
Bony interorbital width	0.256	0.018
Premaxilla length	0.222	0.258
% of total variance	97	1.0

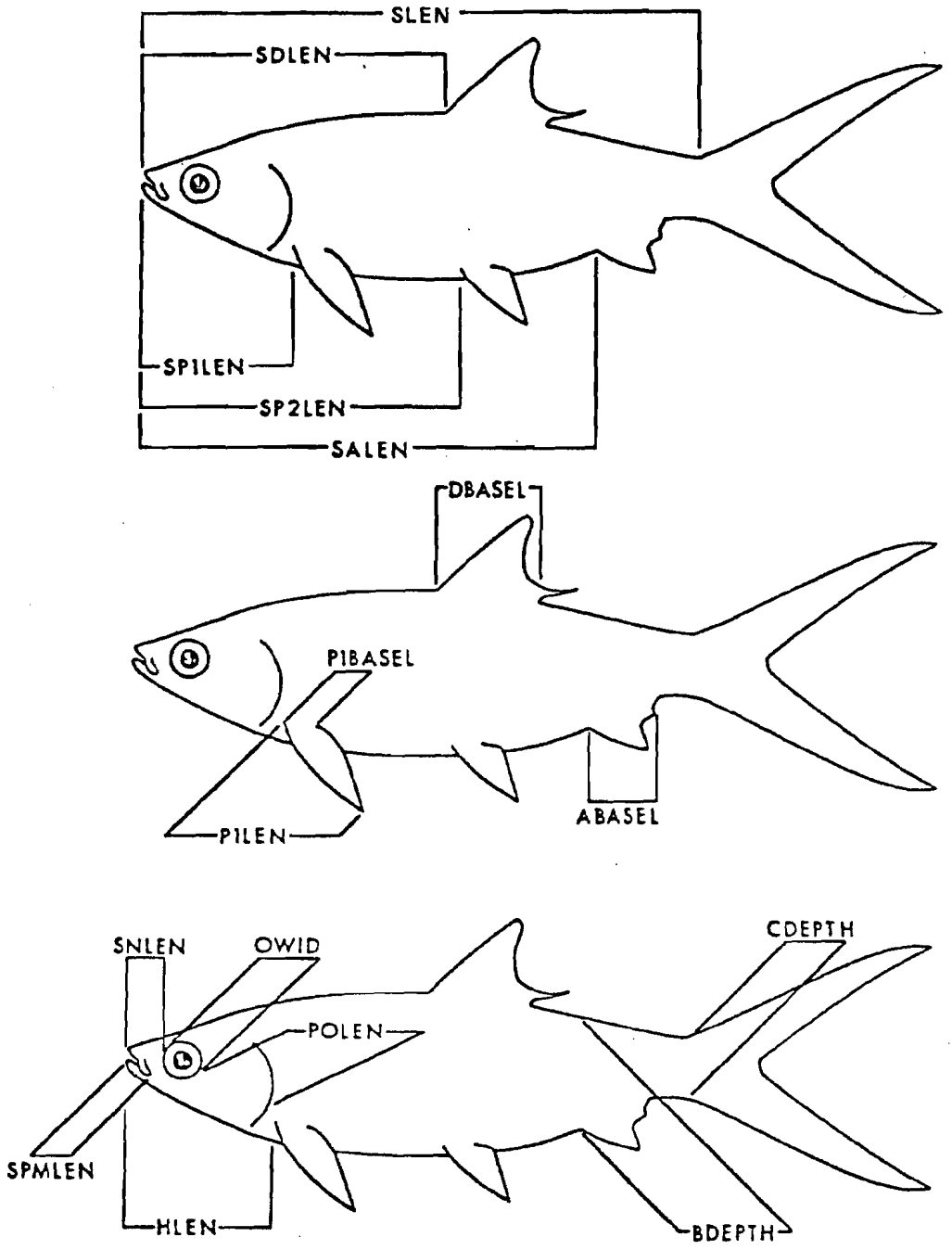

Figure 1. Example of conventional morphometric characters in milkfish. Descriptions are given in Table 6.

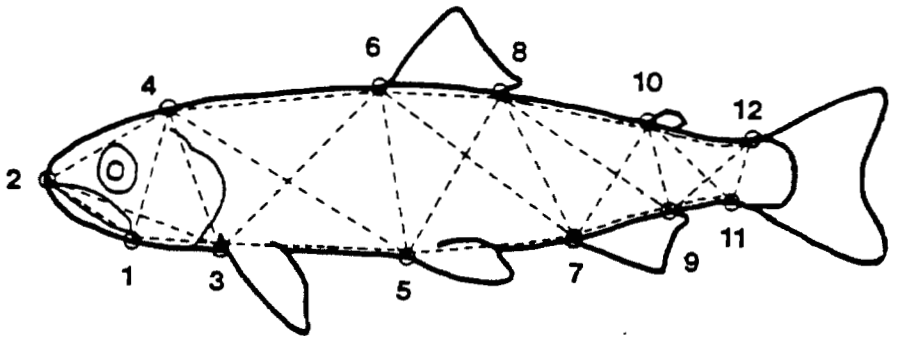

Figure 2. Example of truss network characters. Morphological landmarks are numbered and morphometric distances between landmarks are dashed lines.

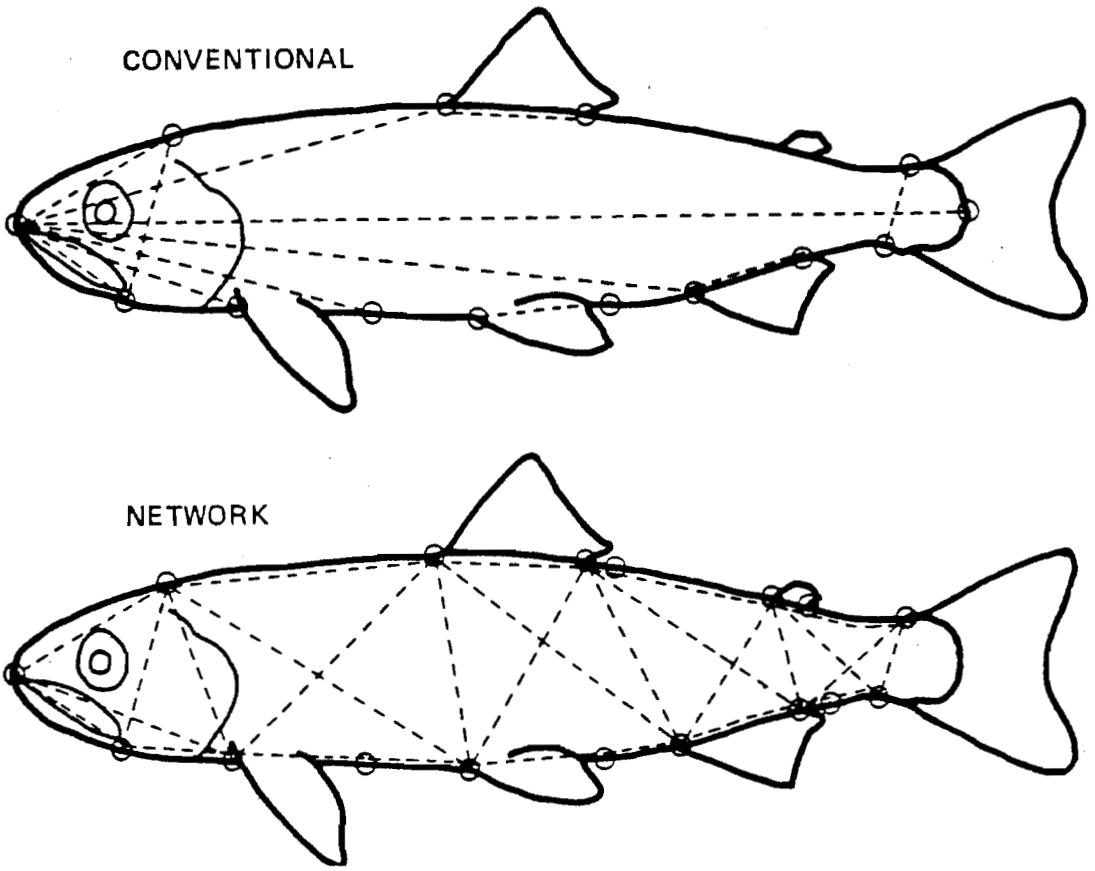

Figure 3. Truss network and conventional characters for chinook salmon.

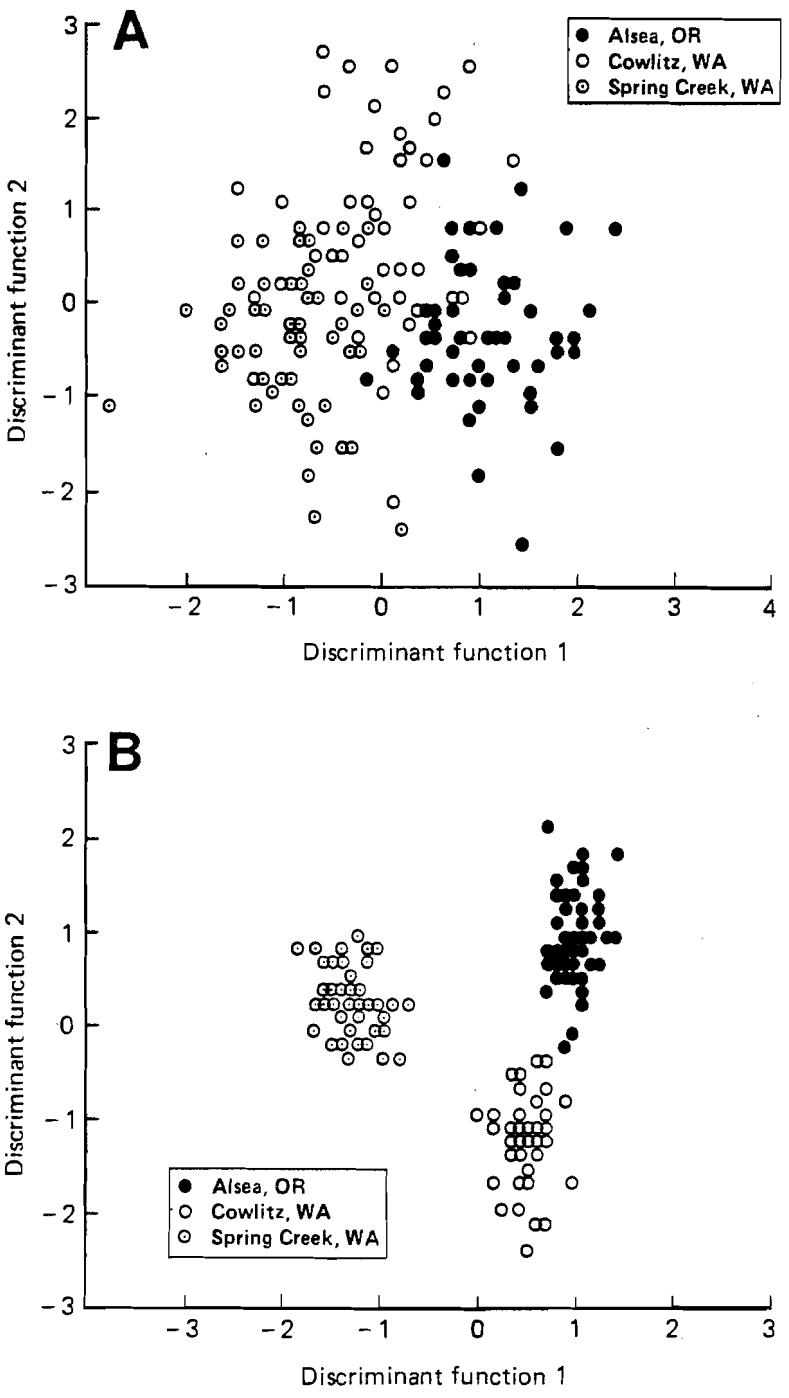

Figure 4. Results of discriminant function analyses of chinook salmon. Analyses are based on conventional measurements (A) or truss network characters (B) illustrated in Figure 3.

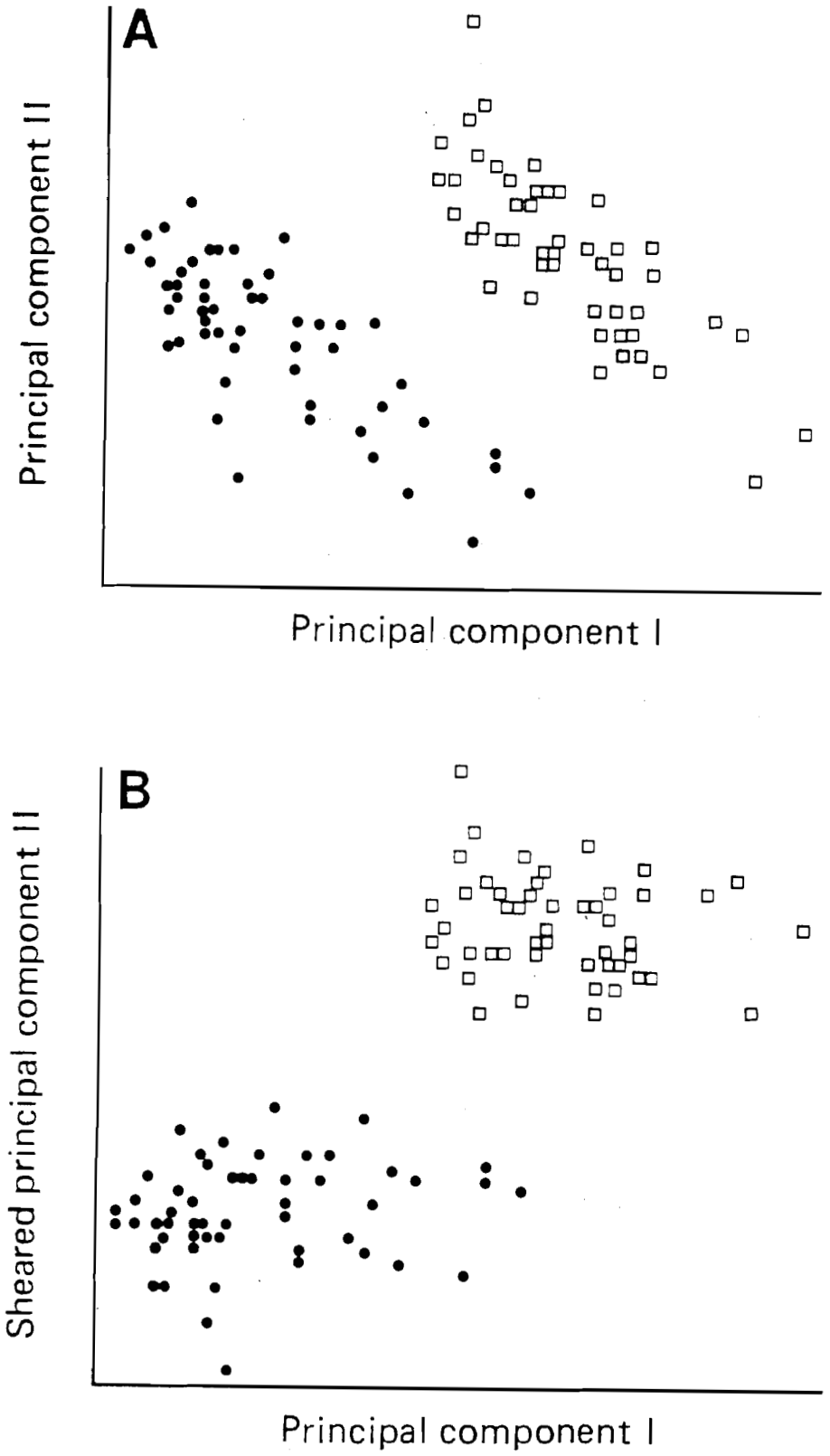

Figure 5. Example of the effects of shear analysis on two samples of chinook salmon. Scatter of points on the first PC axes before (A) and after (B) a shear analysis (Bookstein et al. 1985).

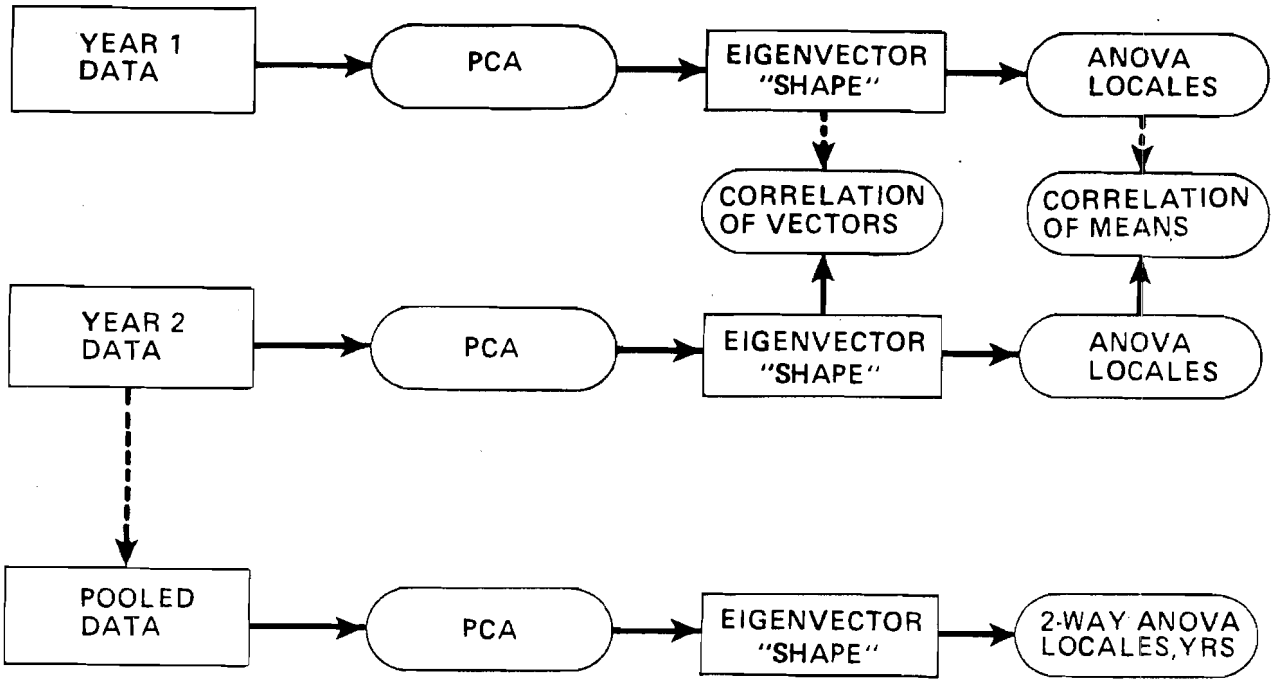

Figure 6. Model for examining temporal stability of morphological variability. PCA = principal component analysis. ANOVA = analysis of variance.

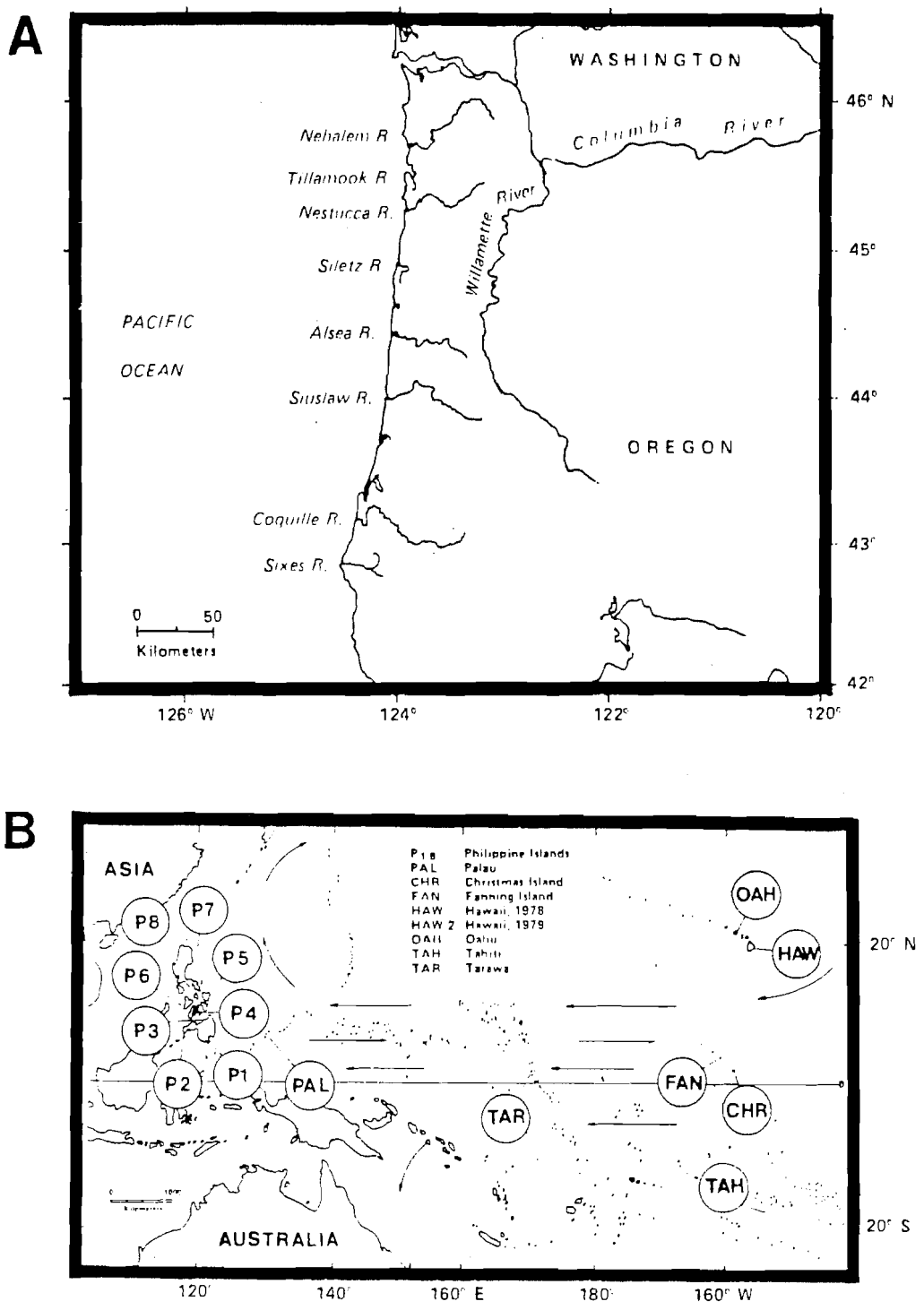

Figure 7. Sampling locations. A. Chinook salmon sampled along the coast of Oregon. B. Milkfish collected from 15 locations in the Pacific Ocean.

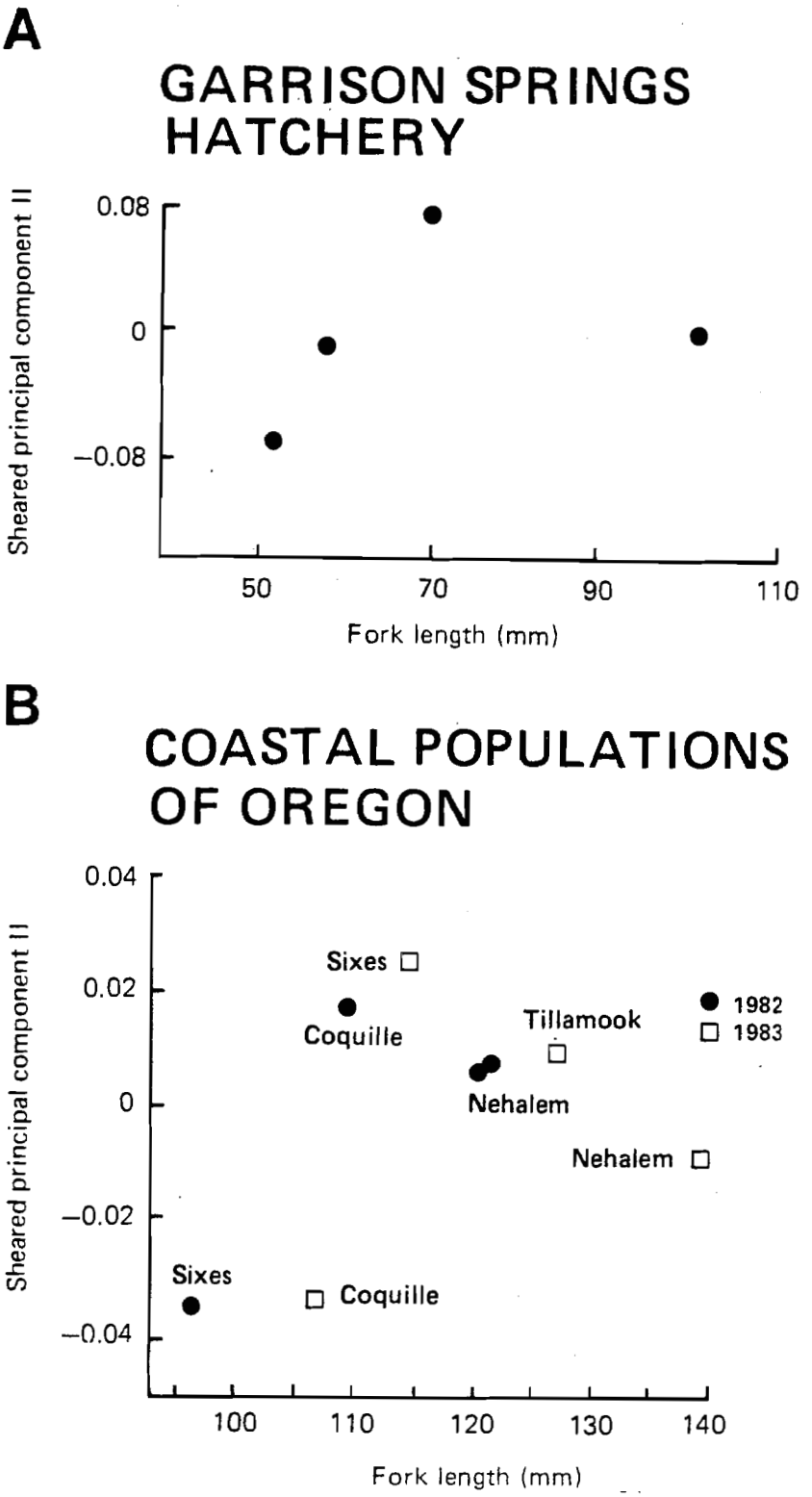

Figure 8. Mean values per sample of chinook salmon for fork length and sheared PC II. Details of the study of chinook salmon from Garrison Springs State Salmon Hatchery, Fort Steilacoom, WA, are given in Winans (1984).

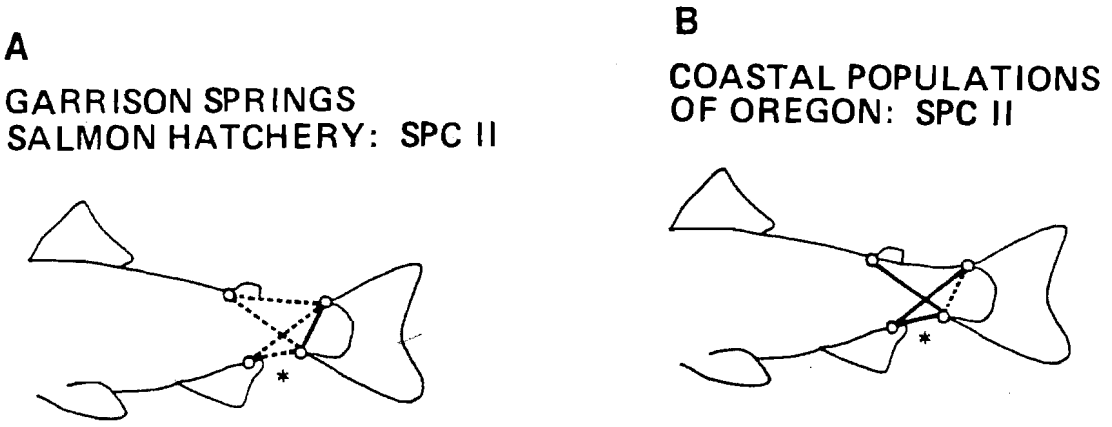

Figure 9. Important characters on SPC II axis. The asterisk indicates the most heavily-weighted character. Negatively signed characters have dashed lines; positively signed characters have solid lines.

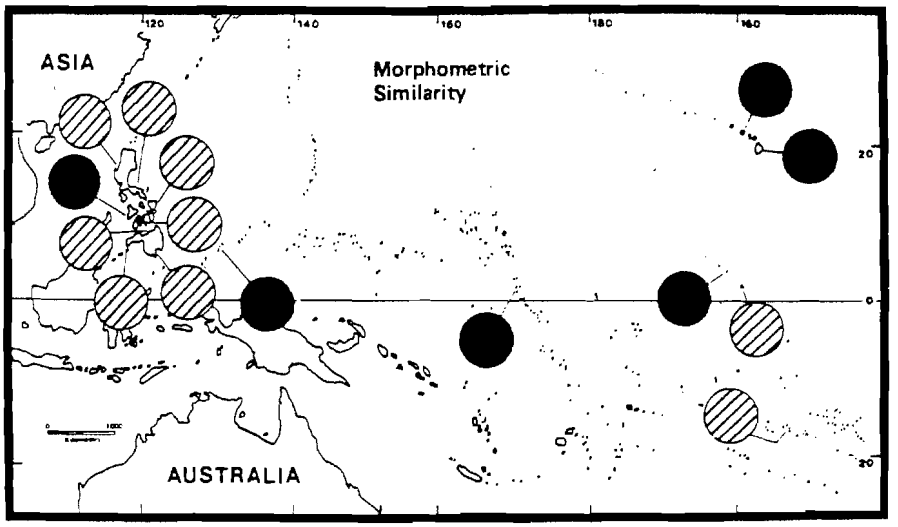

Figure 10. Morphometric similarity of milkfish samples. Solid circles indicate a positive SPC II value and lined circles indicate a negative SPC II value (see Winans 1985).

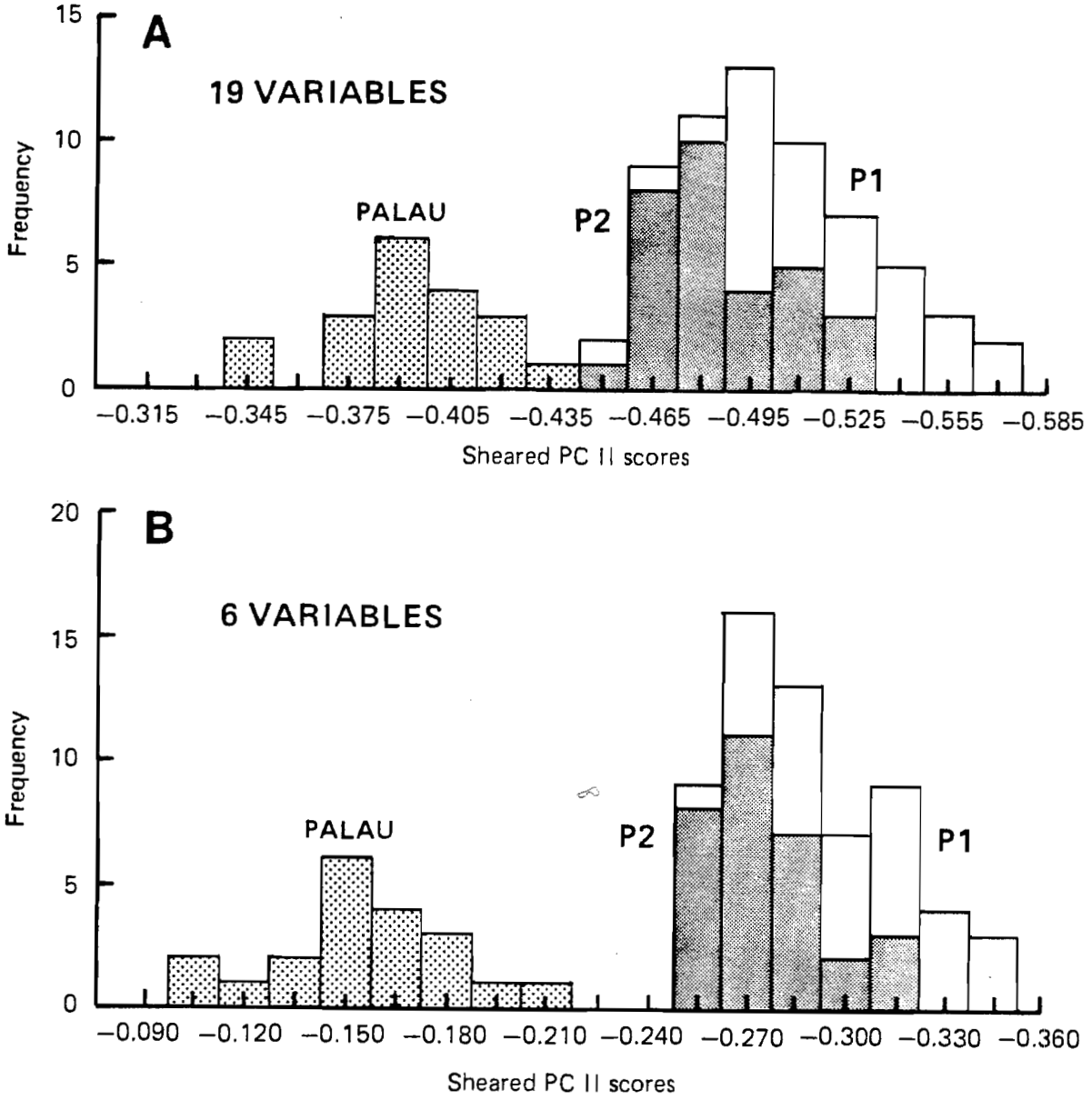

Figure 11. Frequency histogram of SPC II values. SPC II values were calculated with 19 morphometric variables (A) and with the 6 most important variables (B). Samples P1 and P2 are from the Philippines (see Figure 7B).