

## The Effect of Sample Size on the Stability of Principal Components Analysis of Truss-Based Fish Morphometrics

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**Abstract.**—Multivariate analysis of fish morphometric truss elements for stock identification, description of new species, assessment of condition, and other applications is frequently conducted on data sets that have sample sizes smaller than those recommended in the literature. Minimum sample size recommendations are rarely accompanied by empirical support, and we know of no previous assessment of minimum sample sizes for multivariate analysis of fish truss elements. We examined the stability of outcomes of principal components analysis (PCA) of truss elements, a commonly applied method of morphometric analysis for fishes, by conducting PCA on 1,000 resamples for each of 24 different sample sizes ( $N$ ; each sample drawn without replacement) from collections of yellow perch *Perca flavescens* (397 fish), white perch *Morone americana* (208 fish), and siscowet lake trout *Salvelinus namaycush* (560 fish). Eigenvalues were inflated and loadings on eigenvectors were highly unstable for the first three principal components (PCs) whenever  $N$  was smaller than the number of truss elements ( $P$ ). Stability of eigenvalues and eigenvectors increased as the  $N:P$  ratio increased for all three species, but the  $N:P$  ratio at which stable results were achieved varied by species. Our results suggest that an  $N:P$  ratio of 3.5–8.0 was required for stability of PC2 and PC3, which is required for analysis of fish shape. Because some of our results varied among the species we examined, we recommend similar evaluations for other species. Results from past work that used PCA of truss elements and where  $N$  was less than  $P$  may require re-evaluation.

Fish morphometrics are studied extensively in fishery science. A common application is to support descriptions of new species based on morphological dissimilarity to closely related species (e.g., Crabtree 1989; Creech 1992; Humphries and Cashner 1994; Stauffer and van Snik 1997; Teugels et al. 2001; Ingenito and Buckup 2005; Devaere et al. 2007; Welsh and Wood 2008). Some studies are taxonomic redescrptions that include analysis of morphometrics (e.g., Fink 1993; Bestgen and Propst 1996; Das and Nelson 1996; Vreven and Teugels 2005). Morphological analysis is also used to study phylogeny (Morrison et al. 2006), phenotypic plasticity (Hard et al. 1999; Gillespie and Fox 2003), fish condition (Smith et al. 2005), and differences among stocks or morphotypes (e.g., Kinsey et al. 1994; Bronte et al. 1999; Moore and Bronte 2001; Alfonso 2004; Hoff 2004; Zimmerman et al. 2006) and to determine parental species of purported hybrids (Taylor et al. 1986; Bostrom et al. 2002). In the Pacific

Northwest, researchers used morphological analysis to describe changes associated with smoltification of anadromous salmonine fishes (Winans and Nishioka 1987) and to nonlethally predict when smoltification has occurred (Beeman et al. 1994). Morphological analysis has also been used to identify unique stocks of endangered cyprinids (Douglas et al. 1989) and to assess sexual dimorphism (Douglas 1993).

Since publication of Bookstein et al. (1985), the collection and analysis of fish morphometrics has been dominated by truss measurements analyzed using multivariate statistics (hereafter, multivariate morphometrics). Traditional methods of collecting fish morphometrics (e.g., Hubbs and Lagler 1964) relied on numerous measurements along the longest axis of the body and thus failed to capture other aspects of shape (Bookstein et al. 1985). An advantage of the truss method promoted by Bookstein et al. (1985) is that the method is not dominated by redundant measurements along a single axis and thus provides a more complete characterization of shape. These data are commonly analyzed using multivariate statistics, such as principal components analysis (PCA), which is superior to

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univariate analyses because PCA considers the covariance or correlation structure of the data and simultaneously considers relationships among all measures rather than abstracting individual morphometrics (Bookstein et al. 1985).

The use of geometric morphometrics (GM) methods and analyses (Zelditch et al. 2004), such as the thin-plate spline (Bookstein 1991), to analyze fish morphometrics has increased over the past decade, but multivariate morphometrics is still commonly used for most of the applications previously cited. Several recent comparisons of multivariate morphometrics with the thin-plate spline method (Douglas et al. 2001; Parsons et al. 2003; Trapani 2003; Busack et al. 2007) or with relative warp analyses (Sidlauskas et al. 2006) have demonstrated that neither is superior to multivariate morphometrics in terms of ability to distinguish morphometric differences among species or groups. Douglas et al. (2001) and Parsons et al. (2003) reported that it is easier to visualize shape differences using outcomes of thin-plate spline analyses. Trapani (2003) and Parsons et al. (2003) also reported greater ability to discriminate more subtle morphometric differences among groups using thin-plate splines. Richtsmeier et al. (2002) discussed many of the assumptions, benefits, and liabilities of multivariate morphometrics and GM.

In practice, the first principal component (PC1) of morphometrics is interpreted as a size axis when variable loadings are similar in magnitude and sign (Bookstein et al. 1985), and the second (PC2) and third (PC3) principal components are interpreted as shape variables (for detailed discussions of size and shape in multivariate examinations of morphology, see Humphries et al. 1981, Bookstein 1989, and Sundberg 1989). The magnitudes of loadings, regardless of sign, determine the influence that a particular morphometric variable has on the principal component. A high-magnitude loading indicates that a morphometric variable is influential. Variation explained beyond PC3 is usually low; hence, the remaining components are typically ignored. In practice, differences among groups in the magnitude of loadings of a morphometric variable on a principal component are interpreted as differences in that trait. Although statistically rigorous methods are available for determining the significance of eigenvalues (Grossman et al. 1991) and loadings (Peres-Neto et al. 2003), a common practice is to select an absolute threshold value above which a morphometric variable is considered significant. Parsons et al. (2003) reported that a commonly applied minimum threshold for importance of a loading is an absolute value of 0.3.

A common problem with many fish morphology studies that use multivariate analysis is potentially

inadequate sample size, either in terms of number of fish ( $N$ ) examined or  $N$  relative to the number of parameters ( $P$ ; i.e., number of truss elements). Johnson (1981) recommended a minimum  $N:P$  ratio of 3–5 plus an additional 20 fish, while Hair et al. (1987) recommended an  $N:P$  ratio of 4 (McGarigal et al. 2000). Neither recommendation was supported with empirical data but relied on theoretical arguments. Johnson (1981) argued that large samples are necessary to adequately capture variation and to overcome difficulties arising from violations of assumptions of multivariate methods. Hair et al. (1987) argued that overfitting is less likely with larger sample-to-variable ratios. Grossman et al. (1991) demonstrated using published data that statistical differences among eigenvalues of principal components are reliable when  $N:P$  is 3 or greater, but they did not evaluate any other outcomes of PCA. Several minimum values of  $N$  are recommended for multivariate analyses of psychometric data (e.g.,  $N = 50$ : Barrett and Kline 1981;  $N = 100$ : Gorsuch 1983;  $N = 250$ : Cattell 1978) but again most are not supported empirically. To our knowledge, there have been no evaluations of minimum  $N$  for multivariate analysis of fish morphometrics. Small  $N$ -values may fail to adequately capture covariance or morphological variation, which may lead to false conclusions regarding differences among groups (McGarigal et al. 2000).

To determine the prevalence of small  $N$  or  $N:P$ , we reviewed 85 papers selected randomly from among 234 papers that cited Bookstein et al. (1985). These studies included taxonomic revisions, new species descriptions, and studies of niche relationships, hybridization, phylogeny, phenotypic plasticity, and other objectives for 35 families of fishes. Sixty-eight percent of the 99 PCAs reported in these papers had  $N:P$  values less than 10, 31% had  $N:P$  values less than 3, and 6% used fewer fish than the number of measurements made ( $N:P < 1$ ). Based on these results, we examined the effect of a range of  $N$  and  $N:P$  ratios on results of analyses of comparatively large truss element data sets describing three fish species from the Laurentian Great Lakes. Our objectives were to examine the stability of results of multivariate analysis of truss element data for a broad range of  $N$  and  $N:P$  and to recommend a minimum  $N$  or  $N:P$  for PCA of truss elements.

## Methods

*Truss element data.*—We collected truss element data from yellow perch *Perca flavescens* collected from the central basin of Lake Erie, white perch *Morone americana* collected from the western basin of Lake Erie, and siscowet lake trout *Salvelinus namaycush* (hereafter, siscowet) collected from Lake Superior. The yellow perch is a valuable recreational and commercial

species in Lake Erie, and assessing morphological differences among purported stocks could be used to assist its management. The white perch is an invasive species in Lake Erie that became very abundant following its establishment in the late 1970s. The siscowet is one of three principal morphotypes of lake trout still present in Lake Superior (Lawrie and Rahrer 1973; Moore and Bronte 2001). Yellow perch (397 fish) were collected in bottom trawl surveys in May 2007. White perch (208 fish) were collected in bottom trawls during assessment surveys throughout the western basin of Lake Erie in 2006. Siscowet (560 fish) were collected with large-mesh (114–152-mm stretch measure), bottom-set gill nets for a study on morphological variation (Bronte and Moore 2007). All fish were held on ice for no more than 24 h before the collection of morphological data.

For yellow perch and white perch, images were captured with a digital camera and imported into SigmaScanPro version 5.0.0 software (SPSS, Inc., Chicago, Illinois). Siscowet images were captured on film, and the images were then digitized and imported into SigmaScanPro for collection of truss elements (Bronte and Moore 2007). Cartesian coordinates (*X*, *Y*) were identified for morphological features (e.g., fin origins and insertions and tip of snout) following the truss method (Bookstein et al. 1985), and distances were measured between them. This resulted in 22 truss elements each on yellow perch and white perch and 32 truss elements on siscowet.

*Statistical analyses.*—We used resampling to assess the effect of a range of *N* and *N:P* on the results of PCA on fish truss elements. We randomly selected 1,000 subsamples of fish without replacement for each of 24 different *N:P* ratios ranging from 0.2 to 10.0 (Table 1) for each species. The maximum possible *N:P* ratio depended on the number of fish and truss elements available for each species. Maximum *N:P* was 18 for yellow perch and siscowet and 9.9 for white perch (Table 1). We only examined *N:P* ratios up to 10 for yellow perch and siscowet because 10 is the maximum ratio recommended in the literature and because morphological studies of fish rarely exceed that ratio. Principal components analysis was conducted on each subsample using the covariance matrix of the log<sub>10</sub> transformed measures. All analyses were conducted using the PRINCOMP procedure in SAS/STAT version 9.1 of the Statistical Analysis System for Microsoft Windows.

We examined four measures of stability from the results of PCA among the various *N:P* ratios for each species: (1) the suitability of PC1 as the size parameter, (2) the variation explained by the first two shape parameters, (3) the stability of the principal component

TABLE 1.—Subsample sizes used in resamples of yellow perch, white perch, and siscowet lake trout for principal components analysis of morphometric truss elements based on a target ratio of 0.2–10.0 for sample size *N* to the number of parameters *P* (*N:P*).

Target ratio ( <i>N:P</i> )	Yellow perch <i>N</i>	White perch <i>N</i>	Siscowet <i>N</i>
0.2	4	4	6
0.3	7	7	10
0.4	9	9	13
0.5	11	11	16
0.6	13	13	19
0.7	15	15	22
0.8	18	18	26
0.9	20	20	29
1.0	22	22	32
1.2	26	26	38
1.4	31	31	45
1.6	35	35	51
1.8	40	40	58
2.0	44	44	64
2.5	55	55	80
3.0	66	66	96
3.5	77	77	112
4.0	88	88	128
5.0	110	110	160
6.0	132	132	192
7.0	154	154	224
8.0	176	176	256
9.0	198	198	288
10.0	220	—	320

loadings, and (4) the identification of individual truss elements that define fish shape. Variation in the sign and the magnitude of the loadings of PC1 was used as a measure of the suitability of PC1 as the size parameter. The PC1 is assumed to be a general representation of fish size if the loadings are the same sign and similar in magnitude (Bookstein et al. 1985). Variation (var) in the sign of the loadings of PC1 was characterized by the mean of the SD (among loadings) of a sign indicator variable:

$$\frac{\sum_{h=1}^m \sqrt{\text{var}_h(p_{hi})}}{m}$$

where

$$p_{hi} = \begin{cases} 1 & \text{if } l_{hi} > 0 \\ 0 & \text{otherwise,} \end{cases}$$

*l<sub>hi</sub>* are the *i* loadings for PC1 in resample *h*, and *m* is the total number of resamples (1,000). Variation in the magnitude of the loadings of PC1 was similarly characterized by the mean of the SD of the loadings for PC1:

$$\frac{\sum_{h=1}^m \sqrt{\text{var}_h(l_{hi})}}{m}$$

Eigenvalues of PC2 and PC3 were used as a measure of the variation explained by the first two shape parameters. The first eigenvalue represents the maximum variation in the sample that can be described by one dimension, typically attributed to size; the next two eigenvalues represent the maximum variation remaining in the sample that can be described by two orthogonal dimensions, typically attributed to shape. We report the ratio of the sum of the second and third eigenvalues to the first eigenvalue.

Correlation among the principal component loadings from different resamples was used to measure their stability. For each  $N$ -value and each of the first three principal components, the mean absolute correlation (MAC) for all possible pairs of resamples was calculated as

$$MAC_k = \frac{\sum_{j=1}^g |r_{jk}|}{g},$$

where  $r_{jk}$  is the correlation of loadings between the  $j$ th pair of resamples for  $PC_k$  and  $g$  is 499,500, the number of all possible pairings of 1,000 random resamples. A high MAC value indicates that the loadings for the different truss elements are similar across all resamples and that the subset of metrics perceived as important in defining this feature are consistent from one sample to the next. We chose a MAC of 0.71 as an informative threshold, indicating the point at which loadings from one resample describe 50% of the variation in any other resample on average. A low MAC means that the loadings vary among resamples and that the subset of metrics perceived as important in defining this metric change from one sample to the next.

The proportion of times each truss element loaded greater than 0.3 in absolute value (following Parsons et al. 2003) for PC2 or PC3 was used to identify individual truss elements that define fish shape. We plotted these proportions against  $N:P$  for each species to visually display how interpreted importance of truss elements varied with  $N:P$  and  $N$ .

**Results**

Variation in the sign and the magnitude of the loadings for PC1 decreased with increasing  $N$  and  $N:P$  (Figure 1). The magnitudes of loadings for PC1 became more similar as  $N$  and  $N:P$  increased, but the pattern differed among species. For all three species, the sign of PC1 was not always the same when  $N$  was less than  $P$ . The sign became uniform at an  $N:P$  of 0.8 ( $N = 18$ ) for yellow perch, 0.9 ( $N = 20$ ) for white perch, and 0.6 ( $N = 19$ ) for siscowet. The maximum SD, whether for sign or magnitude of loadings for PC1, was

related to overall  $N$ ; it was highest for white perch, intermediate for yellow perch, and lowest for siscowet.

The variation explained by the first two shape parameters (PC2 and PC3) stabilized as  $N$  and  $N:P$  increased (Figure 2). Eigenvalues were biased high and were highly variable at low  $N$  and low  $N:P$ , which indicated that variance attributable to differences in shape were overestimated when  $N$  and  $N:P$  were low. Two species showed a step-shift to a lower level of variation explained by shape variables relative to PC1 as  $N$  and  $N:P$  increased. This shift was most apparent for siscowet, still noticeable for white perch, and barely visible for yellow perch and occurred at an  $N:P$  ratio of approximately 1.5.

The stability of loadings for PC1, PC2, and PC3 also increased as  $N$  and  $N:P$  increased, eventually becoming asymptotic (Figure 3). For yellow perch, the loadings began to stabilize (i.e.,  $MAC > 0.7$ ) at an  $N:P$  ratio of 3.5 ( $N = 77$ ) for PC1, 0.5 ( $N = 11$ ) for PC2, and 1.4 ( $N = 31$ ) for PC3. For white perch, the loadings began to stabilize at an  $N:P$  of 3.5 ( $N = 77$ ) for PC1, 1.0 ( $N = 22$ ) for PC2, and 4.0 ( $N = 88$ ) for PC3. For siscowet, the loadings began to stabilize at an  $N:P$  of 0.7 ( $N = 22$ ) for PC1, 2.0 ( $N = 64$ ) for PC2, and 5.0 ( $N = 160$ ) for PC3.

Each species had four to six truss elements that consistently loaded heavily on PC2 and PC3. The proportion of their loadings exceeding 0.3 increased as  $N:P$  and  $N$  increased (Figure 4). These truss elements had loadings greater than 0.3 in 50% of resamples at an  $N:P$  ratio of 2.5 ( $N = 55$ ) for yellow perch, 0.5 ( $N = 11$ ) for white perch, and 3.5 ( $N = 112$ ) for siscowet (Figure 4). Thus, there was at least a 50% chance of identifying the most influential truss element at these levels of  $N:P$  and  $N$ . Each species also had at least one truss element with loadings greater than 0.3 in 50% of resamples at low  $N:P$ , but the proportion of their loading exceeding 0.3 decreased as  $N:P$  and  $N$  increased. All truss elements with a declining proportion of loading greater than 0.3 did so in less than 50% of resamples at an  $N:P$  of 1.0 ( $N = 22$ ) for yellow perch, 7.0 ( $N = 154$ ) for white perch, and 8.0 ( $N = 256$ ) for siscowet (Figure 4), which means there was less than a 50% chance of misidentifying an important truss element at these  $N:P$  ratios and  $N$ -values. There was no common  $N:P$  or  $N$  for stability of loadings for PC2 and PC3 even between yellow perch and white perch, which had the same  $P$ .

**Discussion**

Our results offer several insights on recommended minimum  $N$  or  $N:P$  when conducting PCA of truss elements. For the three species we investigated, a minimum  $N$  of 50, as recommended by Barrett and Kline (1981), was sufficient to achieve stability of eigenvalues of principal components but insufficient to

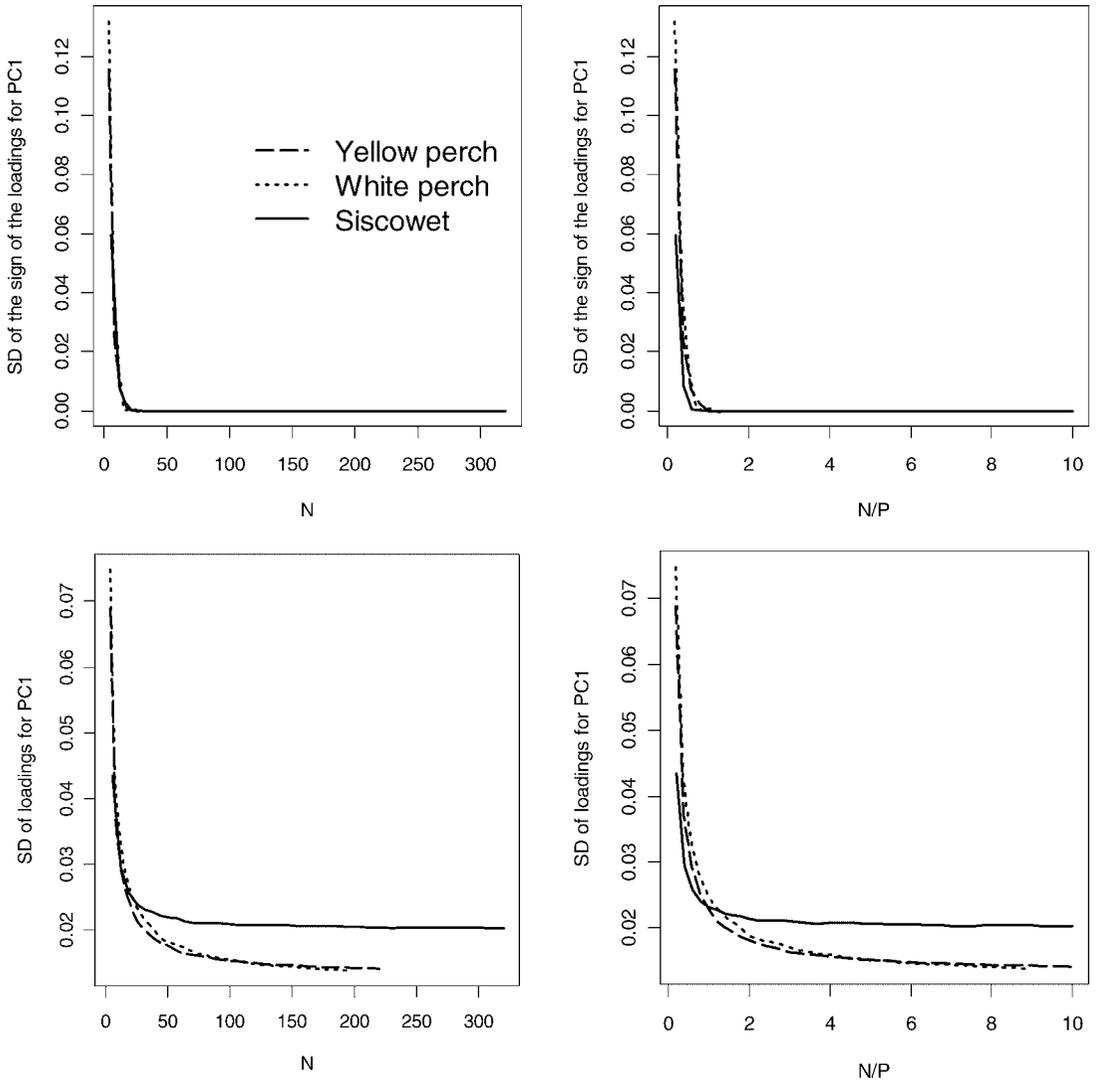


FIGURE 1.—Variation (SD) in the sign (upper plots) and magnitude (lower plots) of the loadings of principal component 1 (PC1) in principal components analysis of morphometric truss elements from yellow perch, white perch, and siscowet lake trout based on 1,000 resamples for each sample size  $N$ . Sample effort is represented as  $N$  (left plots) and as the ratio of  $N$  to the number of parameters  $P$  ( $N:P$ ; right plots).

achieve stability of loadings for the principal components. A minimum  $N$  of 100 (Gorsuch 1983) achieved stability of loadings for PC1–PC3 for all three species (except PC3 for siscowet). For  $N:P$  between 0.6 and 0.9 ( $N < 14$  for yellow perch and white perch;  $N < 29$  for siscowet), the sign of loadings for PC1 was not stable, the variation explained by PC2 and PC3 was inflated, and the loadings were not stable (consistent with the results of Karr and Martin 1981), all of which greatly reduce the reliability of interpreting shape differences. For an  $N:P$  of 3.0 (Johnson 1981), the variation explained by PC2 and PC3 had stabilized for all three

species, but the loadings had not yet stabilized for PC1 for yellow perch, PC1 and PC3 for white perch, and PC3 for siscowet. For  $N:P$  of 5.0 (Johnson 1981), the loadings had stabilized for PC1–PC3 for all three species, but selection of specific truss elements contributing to shape had not yet stabilized for white perch or siscowet (both had more than a 50% chance of misidentification of an important truss element). For an  $N:P$  of 10.0, selection of specific truss elements contributing to shape had stabilized for all three species.

These results suggest that a minimum  $N:P$ , rather than minimum  $N$ , is a superior standard for multivariate

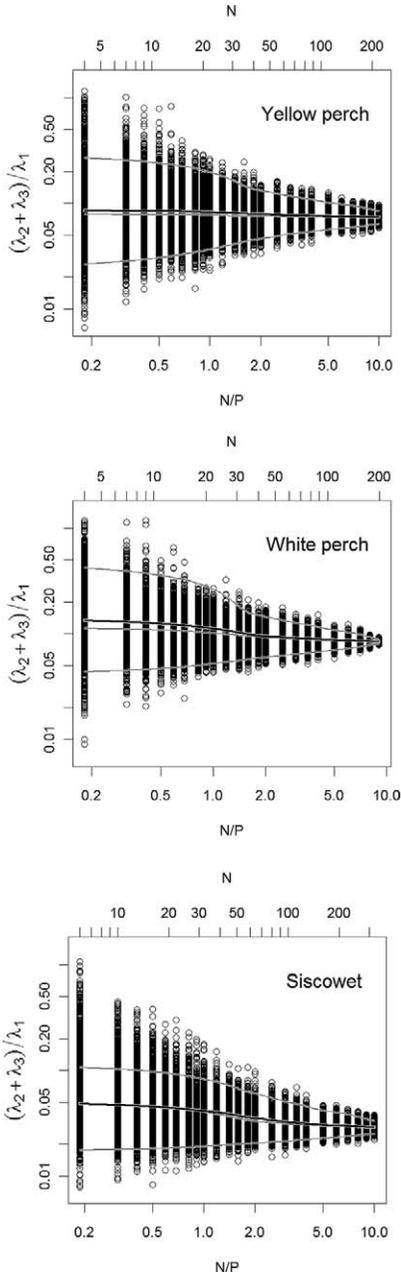


FIGURE 2.—Variation in the first two shape principal components related to sample size  $N$  based on 1,000 resamples for each  $N$  in principal components analysis of morphometric truss elements from yellow perch, white perch, and siscowet lake trout. Plots are on the log–log scale. Variation is expressed as the ratio of the sum of the second and third eigenvalues ( $\lambda_2 + \lambda_3$ ) to the first eigenvalue ( $\lambda_1$ ). All vertical axes are on the same scale. Sample effort is represented as  $N$  and as the ratio of  $N$  to the number of parameters  $P$  ( $N:P$ ). The thick line represents the mean; thin lines represent the median and the 95% quantile range.

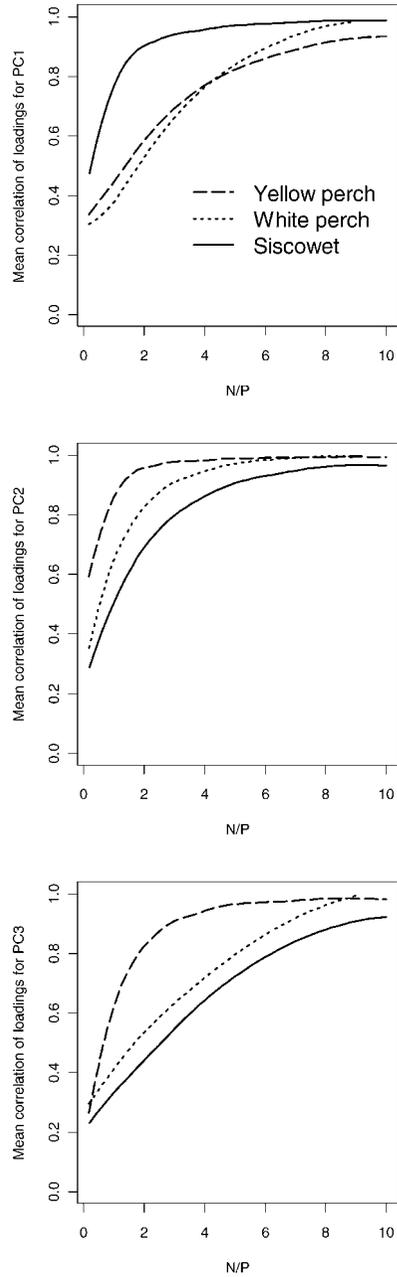


FIGURE 3.—Stability of loadings represented by the mean absolute correlation among loadings for the first three principal components (PC1–PC3) from all possible pairs of 1,000 resamples in principal components analysis of morphometric truss elements from yellow perch, white perch, and siscowet lake trout. All vertical axes are on the same scale. Sample effort is represented as the ratio of sample size  $N$  to the number of parameters  $P$  ( $N:P$ ).

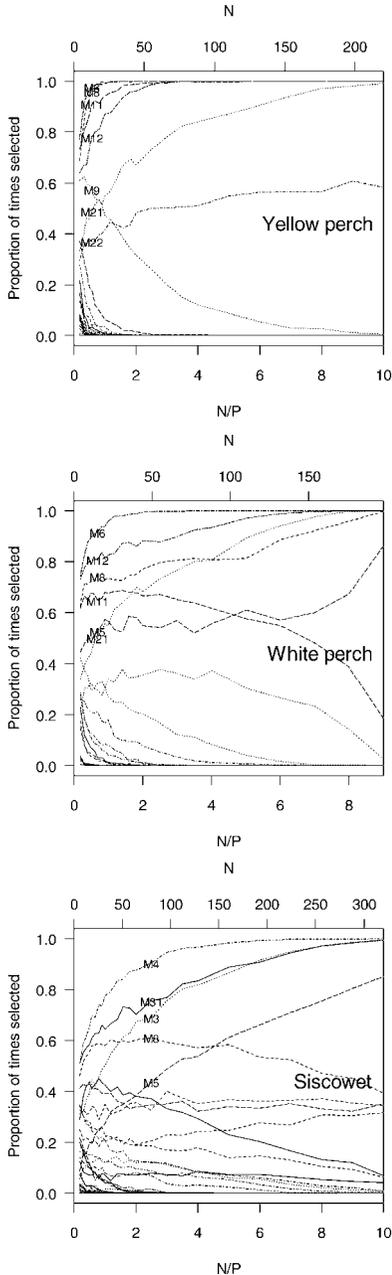


FIGURE 4.—Proportion of times each morphometric truss element from three fish species was selected as an important shape variable (defined as having an absolute loading  $>0.3$  for principal component 2 or 3) related to sample size  $N$  or to the ratio of  $N$  to the number of parameters  $P$  ( $N:P$ ) based on 1,000 resamples for each  $N$ . Each line represents a different truss element variable; those exceeding 0.5 at some point are identified. Truss elements for white perch are posterior dorsal fin insertion to dorsal caudal fin (M6), isthmus to snout (M12), anterior attachment of ventral membrane from caudal fin to anal fin insertion (M8), posterior dorsal fin insertion to anterior attachment of ventral membrane from caudal fin

analysis of truss elements. The range of minimum  $N$  that ensured that the signs of PC1 loadings were the same was narrow ( $N = 18-20$ ). Sample sizes lower than this range yielded PC1s that did not have loadings of the same sign and magnitude. For all other outcomes, the range of  $N:P$  was less variable across species and responses than was  $N$ . Furthermore, other outcomes of PCA used to assess shape variation required higher standards than an  $N$  of 20 (see following discussion). Sample-to-variable ratio is also more favorable intuitively because  $P$  varies across species.

The minimum  $N:P$  required for fish morphometrics studies using the truss method and PCA depends on specific objectives. To achieve stability of loadings for PC1, an  $N:P$  of 3.5 sufficed for all three species examined. For most studies, stability of PC1 is necessary to ensure that PC1 represents a size component, but such stability is not sufficient for assessing variation in shape. Even for studies that regress truss elements against PC1 to account for size differences among groups (e.g., dos Reis et al. 1990; Bronte et al. 1999; Moore and Bronte 2001), stability of PC1 is necessary. For stability of the eigenvalues of PC2 and PC3, which measure variation explained by the first two shape parameters,  $N:P$  values greater than 1.5 sufficed. This outcome suggests a lower  $N:P$  than that recommended by Grossman et al. (1991;  $N:P > 3.0$ ); however, stability of the variation explained by shape is not sufficient for most studies. To determine how shape varies among groups, researchers need to determine those morphological features that are weighted most heavily on PC2 and PC3. This requires stability of these loadings, which was achieved at an  $N:P$  between 0.5 and 8.0 for the three species we examined. Thus, using the most inclusive criteria that permit interpretation of shape variation, we recommend an  $N:P$  ratio of 3.5 or greater for yellow perch (based on stability of PC1 loadings; Figure 3), 7.0 or greater for white perch (based on selection of truss elements contributing to shape; Figure 4), and 8.0 or greater for siscowet (Figure 4) for studies that have assessment of

(M21), pelvic fin to isthmus (M11), and anterior dorsal fin insertion to posterior dorsal fin insertion (M5). For yellow perch, variables M6, M8, M11, M12, and M21 are as described for white perch; additional variables are anal fin insertion to anal fin origin (M9), and anal fin insertion to dorsal caudal fin (M22). Variables for siscowet lake trout are posterior end of maxillary to anterior tip of snout (M4), anterior attachment of ventral membrane from caudal fin to insertion of anal fin (M31), top of cranium at midpoint of eye to posterior end of maxillary (M3), anterior tip of snout to origin of pectoral fin (M5), and posterior point of maxillary to origin of pectoral fin (M8).

shape differences as an objective. These recommendations are, of course, based on the standards we applied when assessing stability of eigenvectors. For example, had we chosen a higher MAC as an informative threshold value for correlations among principal component loadings (e.g., 0.9 instead of 0.7, or apparent inflection points), our recommendations for minimum  $N:P$  would have been higher for all three species. Thus, we reiterate that the preceding standards are the minima; researchers assessing species or groups with highly variable morphometrics or those who want to further reduce the probability of spurious outcomes of PCA should use a higher  $N:P$ .

Cardini and Elton (2007) performed a similar evaluation of  $N$  for GM analysis of the skulls of vervet monkeys *Cercopithecus aethiops*. Their results were similar to ours in that what constituted a sufficiently large sample to achieve accurate and precise analyses depended on the outcome of interest. They reported that larger values of  $N$  were required to achieve accurate and precise estimates of mean shape than those for mean size. This is analogous to our result that the stability of eigenvalues, which are estimates of the variation accounted for by shape, could be achieved with smaller  $N:P$  ratios than stability of eigenvectors, which represent the individual truss elements used to interpret shape. The  $N$  and  $N:P$  reported by Cardini and Elton (2007) were smaller than those recommended here. This may reflect differences between taxa (mammals versus fish), the number of morphological variables analyzed, or resampling methods used to generate subsamples (Cardini and Elton 2007 resampled with replacement, whereas we sampled without replacement). It may also be related to the inherent variability of the study material (mammalian skulls versus entire fish bodies) or the ability of GM to better distinguish shape differences when  $N$  or  $N:P$  is small.

Our results support those of Aleamoni (1973) on theoretical grounds that PCA should not be used when  $N$  is less than  $P$  because unstable results are likely, regardless of study objectives. Our analyses demonstrate that  $N$  in studies of fish morphometrics must be large enough to adequately capture the variability of the subject population. Most authors do not explicitly acknowledge this, although there are exceptions (e.g., Busack et al. 2007). Many authors have circumvented this problem by using multiple-group PCA (Thorpe 1988) when several groups—whether species, genders, populations, or some other category—are under investigation. This avoids the immediate problem of  $N$  being less than  $P$  for any particular group, but it does not compensate for an insufficient  $N$  to capture within-group variability. For example, small  $N$  may have contributed to the “idiosyncratic interpretation of shape

variation” in cichlid fishes analyzed using PCA of truss elements by Parsons et al. (2003). Those authors used  $N$ -values of 9, 13, 14, and 15 for four different species of cichlids from which 20 truss measurements were included in analyses. Combined, these four species had a total  $N$  of 51, which yields an  $N:P$  ratio of 2.55 (but  $N:P < 1.0$  for each species). Our results suggest that the combined  $N:P$  may have been insufficient to achieve stable loadings on PC2 and PC3. The GM methods used by Parsons et al. (2003) did not produce the “idiosyncrasies” evident in the analysis using multivariate morphometrics, which suggests that GM methods may be more reliable when  $N$  and  $N:P$  are small. Of course, a thorough analysis of stability of GM outcomes for multiple species of fish will be required before conclusions can be drawn.

The method of resampling from existing data sets probably did not affect our results. Although no individual fish was represented more than once in a single sample (we sampled without replacement), the number of individual fish shared among different samples increased as  $N$  and  $N:P$  increased. For an  $N:P$  of 5.0, two random samples of 110 yellow perch/sample shared 30 fish (28%), two random samples of 110 white perch/sample shared 58 fish (53%), and two random samples of 160 siscowet/sample shared 46 fish (29%) on average. Differences in responses among species suggest that the asymptotic patterns observed were not strongly influenced by increasing similarity of samples. If increasing similarity of samples was forcing the observed asymptotic relationships, then we would have expected a more rapid approach to asymptotes for the smallest data set (white perch) and a slower approach to asymptotes for the largest data set (siscowet). In some cases, the opposite was true; for siscowet, the asymptote was approached at smaller  $N$  and  $N:P$  for stability of eigenvalues for PC1 (Figure 1) and mean correlation of principal component loadings (Figure 3) than for yellow perch and white perch. Asymptotes for mean correlation of principal component loadings were approached soonest for yellow perch on PC2 and PC3 (Figure 3). Had increasing similarity of samples been driving the observed asymptotic relationships, we would have expected asymptotes to be approached soonest for white perch, and this did not occur. Thus, the observed trends are probably reasonable representations of how results of PCA are affected by differences in  $N$  and  $N:P$  ratios.

Our results have implications for past and future work that used or will use multivariate morphometrics to describe fish morphology. First, we propose minimum values of  $N$  based on analyses of empirical data; previous recommendations were general in nature and were derived from theory. Second, our results

demonstrate that minimum  $N$  varies with research objectives; a lesser standard is required for studies that seek to quantify shape variation than for those that seek to describe how shape varies. Third, our results varied by species, which suggests that there is no absolute standard for minimum  $N:P$ . This may be due to the fact that some species are more phenotypically plastic or are evolving more rapidly than others (e.g., African cichlids). Such species would be expected to have more variable morphology and thus will require different standards for minimum  $N$  than others. We recommend similar investigations of other fish species. Fourth, many species descriptions and taxonomic revisions we reviewed that used multivariate morphometrics employed  $N:P$  ratios that were lower than those recommended here, and many used  $N:P$  ratios less than 1.0. Although our results were not consistent for all of the PCA outcomes we evaluated, one consistency was that outcomes were highly unstable whenever  $N:P$  was less than 1.0. Re-evaluation of taxonomic status may be warranted for those species for which multivariate morphometrics were a major factor used in decisions of morphometric dissimilarity and for which  $N:P$  was less than 1.0.

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