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Morphologic comparisons of hatchery-reared specimens of *Scaphirhynchus albus*, *Scaphirhynchus platorynchus*, and *S. albus* × *S. platorynchus* hybrids (Acipenseriformes: Acipenseridae)

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Summary

Extensive habitat modifications within the Mississippi and Missouri rivers have presumably interfered with the reproductive isolating mechanisms between the endangered pallid sturgeon, *Scaphirhynchus albus*, and the sympatric shovelnose sturgeon, *Scaphirhynchus platorynchus*, causing hybridization between these two species. Several character indices were developed to assist fisheries biologists in identifying specimens of *S. albus*, *S. platorynchus*, and their putative hybrids. The indices have numerous assumptions, including that pure strains of both parental species are within the sample analyzed and that hybrids are morphologically intermediate relative to their parents. If these indices have produced inaccurate identifications, then previous work on *Scaphirhynchus* studies in the Mississippi and Missouri rivers are questionable, including status surveys, captive propagation efforts, or the harvesting of tissues for genetic studies. In this study we tested indices by examining progeny of 'known' pallid, shovelnose, and hybrid sturgeon propagated, raised, and preserved at hatcheries. These 60 specimens [78–600 mm standard length (SL)] were propagated with breeding stock from the upper Missouri River drainage, where hybridization between these two species presumably does not occur. Existing indices did not correctly identify small (< 250 mm SL) or a combination of small and large (> 250 mm SL) sizes of *S. albus*, *S. platorynchus*, and their hybrids. Indices worked fairly well in identifying large *S. platorynchus*, but not in differentiating large *S. albus* from hybrids. We used principal components analysis (PCA) as an alternative approach to character indices. No *a priori* knowledge of the identity of the specimen is required with this multivariate technique, which avoids potential circular reasoning present in indices. We were able to completely or almost completely separate both sturgeon species and their hybrids by extracting principal components from a correlation matrix of 13 meristic characters in a standard PCA and extracting size-corrected principal components from a covariance matrix of 51 morphometric variables using a sheared PCA. Additionally, we demonstrated that first generation hybrids were intermediate with respect to their parental species. Multivariate analyses with a reduced character set of six meristic and 12 morphometric variables also led to accurate and reliable specimen identification. Head spines and numerous qualitative characters are also extremely useful in differentiating between *Scaphirhynchus* species and their hybrids. In addition to all morphometric characters, some meristic characters and the degree of head spine fusion vary significantly with SL of sturgeons. Recording appropriate data from released specimens, including photovouchers, and making this information available is essential for researchers to have any scientific or legal basis for genetic or any other studies involving these sturgeons.

Introduction

Scaphirhynchus albus, the pallid sturgeon, is an endangered species ranging from the upper Missouri River in Montana to the lower Mississippi and Atchafalaya rivers in Louisiana (U.S. Fish and Wildlife Service (USFWS), 1990). Extensive habitat modifications have contributed greatly to the demise of this species. Reproduction was reduced or eliminated through destruction or alteration of spawning habitats in the Missouri and middle Mississippi rivers. These same alterations presumably interfered with the reproductive isolating mechanisms between *S. albus* and the sympatric *Scaphirhynchus platorynchus*, the shovelnose sturgeon, causing hybridization between these two species (USFWS, 1993). Intermediates were first reported from samples collected in 1978–1979 in the lower Missouri and middle Mississippi rivers (Carlson and Pflieger, 1981; Carlson et al., 1985). Twelve specimens were classified as hybrids in the field because of their intermediacy for certain characters useful in field identification. A cumulative analytical character index (Carlson and Pflieger, 1981) and principal components analyses (Carlson et al., 1985) using meristic and morphometric characters supported the identification of 75% of these specimens as hybrids. Attempts to corroborate the identity of these specimens as hybrids using protein electrophoresis were unsuccessful; no diagnosable differences were found between *S. albus*, *S. platorynchus*, and the presumed hybrids at 37 gene loci (Phelps and Allendorf, 1983). Additional hybrids were reported from the lower Mississippi River (Warren et al., 1986; USFWS, 1993), indicating that hybridization may occur throughout most of the range of *S. albus*.

Accurate differentiation among specimens of *S. albus*, *S. platorynchus*, and their hybrids in the field is crucial for status surveys, habitat use or migration studies, captive propagation efforts, or the harvesting of tissues for genetic studies. To assist fisheries biologists in field identification, several other cumulative character indices were developed (Keenlyne et al., 1994; Sheehan et al., 1999; USFWS, 2000; Wills et al., 2002). Each character index uses all or a subset of nine morphometric and four meristic characters identified by

Bailey and Cross (1954) as diagnostic between *S. albus* and *S. platorynchus*. For each character or combination of characters, the most *S. platorynchus*-like value from all specimens examined receives a score at one end of a scale. Likewise, the most *S. albus*-like value receives a score at the opposite end of the scale, and all other specimens receive scores somewhere in between. All scaled values for each character for each specimen are then summed or averaged to produce a single scaled value for each specimen. A plot of these scaled values produces a bimodal distribution for *S. platorynchus* and *S. albus*, respectively, with any specimens residing at or near the middle of the scale (between the two bell-shaped curves) being suspected hybrids.

Potential shortcomings of character indices include the assumptions that a sample contains pure strains of both parental species and that the hybrids are morphologically intermediate relative to the parental species. Neither assumption was demonstrated adequately in any previous morphologic studies of these sturgeon species. Not all fish hybrids exhibit intermediacy between parental species (Leary et al., 1983), and the assumption that *S. albus* × *S. platorynchus* hybrids should be intermediate was questioned (Campton et al., 2000) and has no empirical basis. Additionally, genetically mediated morphologic variation can be expressed differently in fish hybrids relative to either parental species, and some hybrids may have more morphologic variability than either parental species (Wilde and Echelle, 1997). Even if most hybrids have intermediate (or near intermediate) index scores, some specimens may be indistinguishable from either parental species due to a particularly high or low index score. Another drawback to traditional character indices is that the index scale changes depending on the sample used, and as sample sizes increase, the range of the scale increases.

An alternative approach to character indices is principal components analysis (PCA). No *a priori* knowledge of the identity of the specimens is required, which avoids the potential circular reasoning of scaling found within character indices (Neff and Smith, 1979). Carlson et al. (1985) used PCA on a correlation matrix of nine morphometric and five meristic characters. A plot of the first two principal components showed most of the field-identified hybrids isolated between the two parental groups, but one and two specimens of putative hybrids were plotted within *S. platorynchus* and *S. albus*, respectively, and one *S. platorynchus* was within the hybrid group. Although the PCA by Carlson et al. (1985) has fewer assumptions than character indices, it had several flaws. First, meristic and morphometric data were combined into one correlation matrix for analysis, but a correlation matrix is only appropriate for meristic data, a covariance matrix is proper for morphometric variables, particularly if size-adjusted shape differences are of interest (Humphries et al., 1981; Bookstein et al., 1985). Secondly, size differences between specimens were addressed within the PCA by dividing morphometric characters by standard length (SL). But using ratios in a PCA can inflate the first eigenvalue and can change the magnitude and direction of coefficients on the various principal components (Atchley et al., 1976). An alternative approach is to assume that size variation among specimens can be adequately removed on the first principal component, and the remaining components will represent size-free variation (Atchley et al., 1976). But size variation can be present in subsequent components, confounding the actual shape difference between specimens. To overcome this problem the size factor can be 'sheared' from the shape component of the matrix of a PCA, (Humphries et al., 1981; Bookstein et al., 1985), a method adapted here.

Allometry plays another important role in sturgeon morphometrics. Differential growth is apparent in several measurements between small (< 250 mm SL) and large (> 250 mm SL) specimens of *Scaphirhynchus* (Bailey and Cross, 1954). Additionally, more measurements were significantly different between small and large *S. platorynchus* than between either size class and a congener, *Scaphirhynchus suttkusi*, the Alabama sturgeon (Mayden and Kuhajda, 1996). Historically, character indices were used exclusively on adult sturgeon and, because of allometry, are probably not appropriate for small individuals. Recent captures of juvenile *Scaphirhynchus* in the Mississippi River by numerous researchers, however, necessitates the need for accurate identification of small individuals to assist in identifying spawning or nursery sites for *S. albus*.

With the shortcomings of using traditional morphologic characters and existing character indices to identify specimens, serious questions arise about the accuracy of previous identifications of *S. albus*, *S. platorynchus*, and their purported hybrids. Moreover, genetic work on these species often relies on tissues harvested from specimens identified with these character indices; after tissue collection specimens typically are released into the wild without any or minimal vouchering protocol. If tissues are taken from specimens thought to be pure parental species or hybrids, but are actually misidentified, then the entire basis of establishing genetic markers is compromised. A more rigorous examination of morphologic data and the quantification of morphologic variation for species identification may resolve some of the current uncertainty with molecular markers.

Here, we investigate several avenues in an attempt to address identification issues among *S. albus*, *S. platorynchus*, and their putative hybrids. First, we tested existing indices. We reexamined data from Keenlyne et al. (1994) to determine the effect of sample size and geographic scale on their character index. We then tested the accuracy of several character indices (Carlson and Pflieger, 1981; Keenlyne et al., 1994; Sheehan et al., 1999; USFWS, 2000; Wills et al., 2002) with data from *S. albus*, *S. platorynchus*, and *S. albus* × *S. platorynchus* progeny, all propagated and raised at fish hatcheries. We tested indices across a range of sturgeon-size classes. Secondly, we examined the influence of post-mesolarval size on meristic data in small sturgeons. Accurate identification of small *Scaphirhynchus* is vital for detecting successful recruitment for *S. albus*, and meristic data are useful in differentiating small specimens. Thirdly, we used univariate and multivariate (PCA) analyses to investigate their utility in distinguishing among known specimens of *S. albus*, *S. platorynchus*, and their hybrids. Finally, we examined a suite of mensural and qualitative characters to discover additional distinguishing characters and tested the assumption of hybrid intermediacy between parental species.

Materials and methods

The U.S. Fish and Wildlife Service propagated and raised specimens of *S. albus*, *S. platorynchus*, and *S. albus* × *S. platorynchus* hybrids at Miles City State Fish Hatchery, Montana, and Gavins Point National Fish Hatchery, South Dakota. All brood stock was captured in the upper Missouri River drainage in extreme western North Dakota and eastern Montana where hybridization between these two species is not known to occur. Two male *S. albus* and one female *S. platorynchus* were used to create the hybrids. Progeny were preserved in formalin at various times from early in development up to small adults, and later transferred to 70% ethanol. A total of 60 of these specimens (14 pallid, 12 shovelnose, and 34 hybrids) ranging in size from 78 to 600 mm SL (85 to 641 mm fork length, FL) were used in this study. Specimens appeared normal except for the lack of spines on the snout. Snout spines are present in almost all wild-caught pallid and shovelnose sturgeon (Bailey and Cross, 1954; Mayden and Kuhajda, 1996). For brevity, the two parental species of sturgeon and their hybrids are referred to as analytical taxonomic units (ATUs).

Character indices

The effect of sample size and geographic scale on character indices was examined by using data from Keenlyne et al. (1994). In their study specimens of *S. albus* and *S. platorynchus* were examined from three separate reservoirs on the upper Missouri River in Montana and North and South Dakota. Data from the three reservoirs were combined to examine how the index changed if the original study considered a larger geographic scale and all three populations were treated as one population from the upper half of the Missouri River.

The accuracy of four character indices (Carlson and Pflieger, 1981; Keenlyne et al., 1994; Sheehan et al., 1999; USFWS, 2000; Wills et al., 2002) in distinguishing between ATUs was evaluated by analyzing data from up to four meristic and nine morphometric characters from hatchery-reared specimens. The methods of these character indices were followed with the exception of three measurements in the Sheehan et al. (1999) and Wills et al. (2002) index (see below).

Keenlyne et al. (1994) employed a character index using six morphometric characters (head length, mouth width, mouth to inner barbel base, snout to outer barbel base, and inner and outer barbel lengths, standardized by SL). Values for each character were scaled from 0 to 100 based on specimens from their sample, and then summed, with *S. platorynchus*-like parameters on the lower end of the scale.

The USFWS (2000) created a character index similar to that of Keenlyne et al. (1994) except mouth width was not used, FL rather than SL was used to standardize characters, and seven rather than six scores were created and summed. Rather than using minimum and maximum values for each character or set of characters from the sample being analyzed, they provided these values based on 262 specimens from the upper Missouri River. The hatchery samples produced two minimum values below those given, and the formulae were adjusted accordingly.

Carlson and Pflieger (1981) used all six measurements in the Keenlyne et al. (1994) index as well as rostral length (snout to anterior edge of subopercle), orbit length, and tenth lateral plate height. They also included fin-ray counts from dorsal, anal, pectoral, and pelvic fins for a total of 13 characters. Values for each character were scaled from 0 to 1000 based on specimens from within their sample, and then summed and averaged, with *S. platorynchus*-like parameters on the lower end of the scale. Using this index on 30 preserved specimens of *Scaphirhynchus* captured in the Missouri and middle Mississippi rivers, they corroborated the identity of 10 of 12 field-identified hybrids.

The character index developed by Sheehan et al. (1999) and Wills et al. (2002) is based on data derived from the 30 specimens identified by and presented in Carlson and Pflieger (1981). Five measurements (head length, mouth to inner barbel base, snout to outer barbel base, and inner and outer barbel lengths) converted to ratios as well as two counts (dorsal and anal-fin rays) were assigned as independent variables in a multiple regression analysis. The dependent variables were *S. albus*, hybrids, and *S. platorynchus*, each coded as −1, 0, and 1, respectively. Unlike the other character indices, this coding scheme places *S. albus*-like parameters at the lower end of the scale. In our study, the length of the longest barbel rather than the mean length of both barbels was used, and head length was taken with a single measurement rather than with two measurements.

Additional meristic and morphometric characters and analyses

Data were collected and analyzed from an additional 43 morphometric and four meristic characters (52 morphometric and 13 meristic total) in an attempt to evaluate the existing diagnostic characters and to possibly discover other useful differentiating characters. Methods for most counts and measurements follow those of Bailey and Cross (1954), Hubbs

Table 1
Description of 13 meristic characters used in this study

Characters	Description
Dorsal plates anterior to dorsal fin	Post-occipital plate (usually the first dorsal plate with a well formed spine and or keel) posteriad to second pre-dorsal plate; the plate without a keel just anterior to dorsal fin was not counted
Dorsal plates posterior to dorsal fin	Plate lateral to posterior edge of dorsal-fin base posteriad to single dorsal plate at base of caudal fin
Lateral plates	Plate just behind shoulder girdle (even if it was without a ridge) posteriad to last keeled plate
Lateral plates anterior to dorsal-fin origin	Plate which had any part intersected by a vertical line through dorsal-fin origin anteriad to first lateral plate
Ventral–lateral plates	Plate just anterior to pelvic fin anteriad to first keeled plate
Ventral plates between anus and anal fin	Plate lateral to posterior edge of anus posteriad to single preanal plate
Ventral plates posterior to anal fin	Plate lateral to posterior edge of anal-fin base posteriad to single ventral plate at base of caudal fin
Dorsal-fin rays	All anterior rudiments behind pre-dorsal plates; the last ray is split at base
Anal-fin rays	All anterior rudiments behind preanal plates; the last ray is split at base
Pectoral-fin rays	Anterior spine and all posterior rudiments
Pelvic-fin rays	All anterior rudiments
Gill rakers	All structures with ends noticeably free from surrounding tissues on the first arch
Gill raker tips	All structures with ends noticeably free from surrounding tissues of the gill raker

and Lagler (1958), Williams and Clemmer (1991), and Mayden and Kuhajda (1996). Some measurements (fifth dorsal plate and spine) are defined here for the first time. SL and fork length were used to standardize morphometric characters. See Tables 1 and 2 for all character descriptions. Meristic and morphometric data were taken from the left side of specimens except for medial structures and spines. For fin ray counts, as suggested by Bailey and Cross (1954), insect pins were used to mark sectional counts and it was necessary to remove tissue at the base of some fins to count all rudiments.

Neither meristic nor morphometric data met assumptions of normality and equality of variances for any ATU. Therefore, univariate analyses employed the non-parametric Kruskal–Wallis test; *post hoc* tests consisted of pairwise comparisons using the Wilcoxon rank sum test (P-values corrected using the Bonferroni correction). Because the head shape of *S. albus* is different from *S. platorynchus* (Bailey and Cross, 1954), neither head length nor head width was used to standardize the smaller measurements of the head region as in Mayden and Kuhajda (1996); all characters were standardized or regressed

Table 2
Description of 54 morphometric characters used in this study

Characters	Description
Standard length	Tip of snout to posterior edge of last keeled lateral plate
Snout to caudal fork length	Tip of snout to caudal-fin fork
Snout to dorsal-fin origin	Tip of snout to posterior edge of pre-dorsal plate
Snout to pelvic-fin insertion	Tip of snout to anterior base of pelvic fin
Snout to pectoral-fin insertion	Tip of snout to anterior base of pectoral fin
Head length	Tip of snout to bony posterior edge of subopercle
Snout to anterior edge subopercle[1]	Tip of snout to anterior-most edge of subopercle
Snout to tip of spine at head end	Tip of snout to most posterior spine tip at posterior-lateral end of head
Snout to anterior edge of orbit	Tip of snout to anterior edge of bony part of orbit
Snout to anterior edge anterior nostril	Tip of snout to anterior edge of anterior nostril opening
Snout to occiput	Tip of snout to the anterior edge of post-occipital plate
Pectoral-fin to pelvic-fin insertion	Anterior base of pectoral fin to anterior base of pelvic fin
Pectoral-fin length	Anterior base of pectoral fin to distal end of longest fin ray
Pectoral-fin insertion to occiput	Anterior base of pectoral fin to anterior edge of post-occipital plate
Body depth at pectoral-fin insertion	Vertical from venter to dorsum of body, includes ridge or spine of dorsal plate
Head depth just anterior to parietal ridge	Vertical from venter to dorsum of head anterior to parietal ridge
Head depth at anterior edge of anterior nostril	Vertical from venter to dorsum of head at anterior edge of anterior nostril
Pelvic-fin length	Anterior base of pelvic fin to distal end of longest fin ray
Pelvic-fin insertion to anal-fin origin	Anterior base of pelvic fin to posterior edge of preanal plate
Pelvic-fin insertion to dorsal-fin origin	Anterior base of pelvic fin to posterior edge of pre-dorsal plate
Dorsal-fin length	Posterior edge of pre-dorsal plate to distal end of longest fin ray
Dorsal-fin base	Posterior edge of pre-dorsal plate to posterior edge of base of dorsal fin
Anal-fin to dorsal-fin origin	Posterior edge of preanal plate to posterior edge of pre-dorsal plate
Anal-fin origin to last keeled lateral plate	Posterior edge of preanal plate to last keeled lateral plate
Caudal peduncle length	Posterior edge of base of anal fin to posterior edge of last keeled lateral plate
Anal-fin length	Posterior edge of preanal plate to distal end of longest fin ray
Anal-fin base	Posterior edge of preanal plate to posterior edge of base of anal fin
Caudal peduncle depth	Least vertical from venter to dorsum of caudal peduncle
Caudal peduncle width	Width just ventral to lateral ridge or spine at anterior edge of precaudal plate
Tenth lateral plate height	Height measured at tenth lateral plate angle
Fifth dorsal plate and spine length	Anterior edge of fifth dorsal plate to tip of spine on plate
Fifth dorsal plate length	Anterior edge of fifth dorsal plate to posterior base of spine on plate
Fifth dorsal plate and spine height	Ventral edge of fifth dorsal plate to highest point of plate or spine on plate
Fifth dorsal spine height	Dorsal edge of tip of fifth dorsal spine vertical to dorsum of sixth dorsal plate
Interorbital width	Dorsal edge of bony part of left orbit to dorsal edge of bony part of right orbit
Orbit length	Horizontal from anterior to posterior edge of eye
Posterior nostril width	Largest measurement of posterior nostril opening
Anterior nostril width	Largest measurement of anterior nostril opening
Pectoral girdle width	Body width just anterior to anterior base of pectoral fin
Anterior mouth to pectoral-fin insertion	Midline cartilaginous anterior edge of labial depression to base of pectoral fin
Anterior mouth to snout	Midline cartilaginous anterior edge of labial depression to tip of snout
Anterior mouth to base of inner barbel	Midline cartilaginous anterior edge of labial depression to anterior base of inner barbel
Anterior mouth to base of outer barbel	Midline cartilaginous anterior edge of labial depression to anterior base of outer barbel
Anterior mouth to head edge at outer barbel	Midline cartilaginous anterior edge of labial depression to head edge lateral to anterior base of outer barbel
Snout to base of inner barbel	Tip of snout to anterior base of inner barbel
Snout to base of outer barbel	Tip of snout to anterior base of outer barbel
Snout to head edge at anterior mouth	Tip of snout to head edge lateral to cartilaginous anterior edge of labial depression
Outer barbel length	Longest measurement of two outer barbels from anterior base to tip
Inner barbel length	Longest measurement of two inner barbels from anterior base to tip
Head width at outer barbel	Head width at anterior base of outer barbels
Head width at anterior edge of mouth	Head width at cartilaginous anterior edge of labial depression
Head width at tip of spine at head end	Head width at tip of spine at head end, posterior-most if multiple spines present
Head width at widest point	Head width at widest point anywhere on head
Mouth width	Widest measurement on outer edge of lips

[1]The bony structure covering the gill chamber in sturgeons is the subopercle.

with SL for univariate analyses. Sexual dimorphism within ATUs was not explored because specimens were juveniles or small non-reproductive adults. For all analyses SAS (Cary, NC, 1989–1996, 1999) and DataDesk (Ithaca, NY, 1997) were employed using a P-value of $P < 0.05$.

The independence of meristic characters with respect to size was tested by examining the correlation between SL and each meristic character; the Spearman rank correlation coefficient (r_s) was calculated for significant correlations. Characters that varied significantly with size were divided into size classes and analyzed separately. The assumed dependence of morphometric data with size also was examined. Fifth dorsal spine height did not significantly covary with SL, either for small or large specimens across or within ATUs, and was thus dropped from all subsequent analyses. The fifth dorsal plate length did not demonstrate a significant correlation with SL within small *S. albus*; this character along with 11 others did not show significant correlation for large *S. platorynchus*, but these were retained in subsequent analyses because of their covariance with SL within most groups examined.

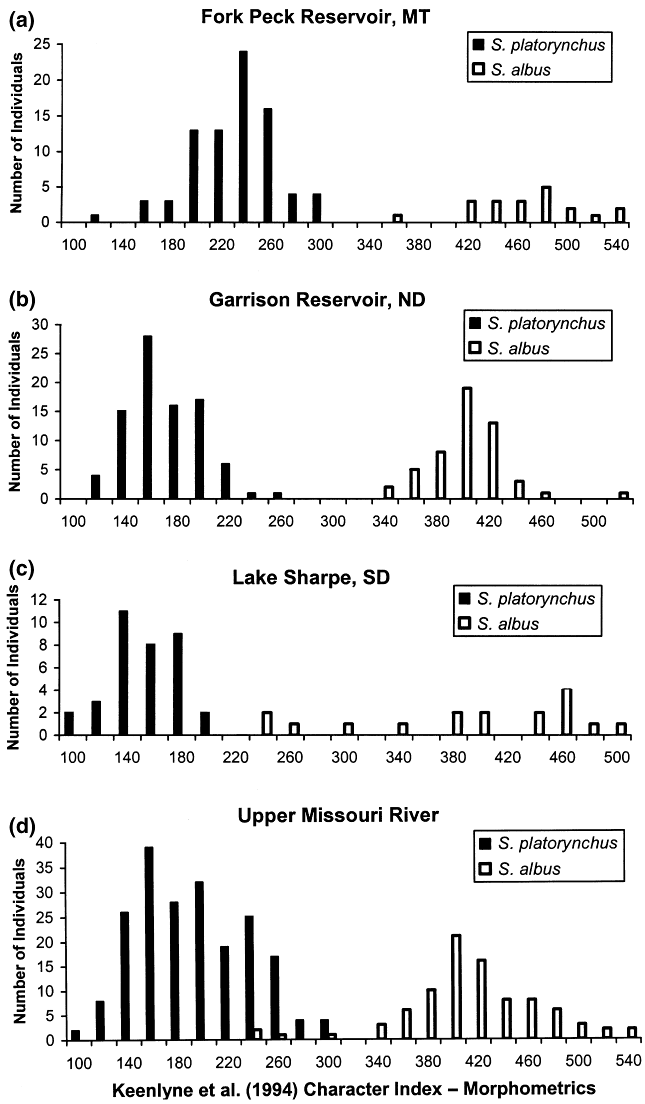

Fig. 1. Character index (Keenlyne et al., 1994) based on morphometric characters for specimens of *Scaphirhynchus platorynchus* and *Scaphirhynchus albus* collected in Missouri River from (a) headwaters above Fort Peck Reservoir, MT, (b) headwaters of Garrison Reservoir, ND, (c) Lake Sharpe, SD, and (d) all data combined for upper Missouri River

Because using SL to adjust size can be problematic as a ratio adjustment or as a covariate in ANCOVA (e.g. Sokal and Rohlf, 1981; Prairie and Bird, 1989; Jackson and Somers, 1991), both methods were used for univariate analyses. Neither arcsine square-root transformed ratios nor log10 transformed raw measurements improved normality and/or homogeneity of variance. Log10 transformed data also did not improve linearity for the ANCOVA, thus untransformed measurements were used for analyses. The homogeneity of slopes assumption of ANCOVA was not met for several morphometric variables: small specimens, pectoral-fin to pelvic-fin insertion, body depth at pectoral-fin insertion, anal-fin length, fifth dorsal plate and spine height, snout to inner and outer barbel bases, and outer barbel length; large specimens, anterior mouth to pectoral-fin insertion. The variability of morphometric ratios between ATUs was compared with the coefficient of variation (CV).

We used PCA as the multivariate analysis to detect morphologic differences between ATUs. Although our data did not meet the assumptions of normality or equal variance, the first few principal components are typically not biased by this deficiency (Legendre and Legendre, 1998). To insure a stable solution in any PCA, the ratio of number of specimens to number of variables should be 3 : 1 or greater (Grossman et al., 1991), but our morphometric data did not meet this ratio (60 : 51). However, the first few eigenvectors are not affected by this shortcoming and should lead to correct interpretations (Legendre and Legendre, 1998). We employed sheared PCA on 51 untransformed characters (D. L. Swofford, SAS Program for computing sheared PCA; unpubl. data, 1984,

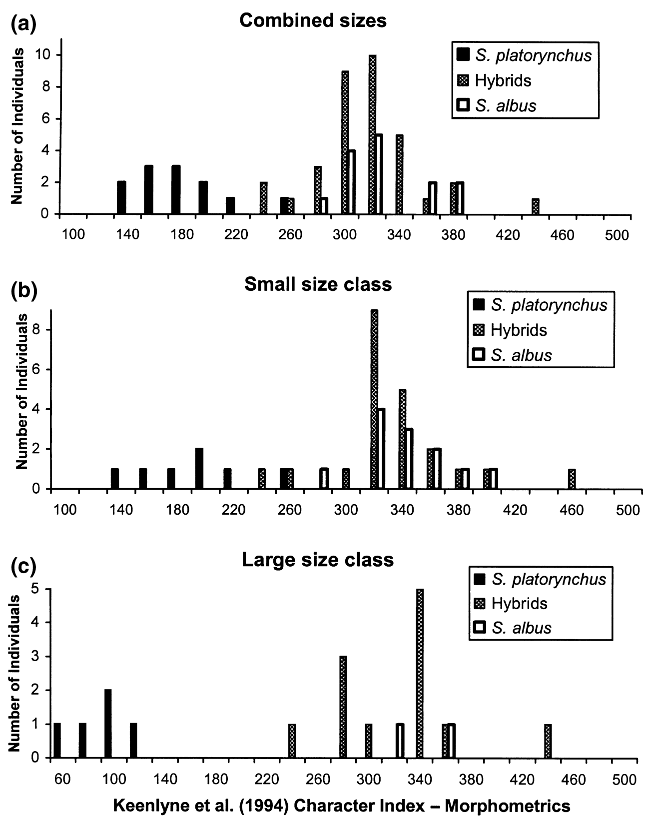

Fig. 2. Character index (Keenlyne et al., 1994) based on morphometric characters for (a) all sizes, (b) small (< 250 mm SL), and (c) large (> 250 mm SL) hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

privately distributed). This method removes size variation among specimens along the first principal component, so that shape differences are expressed along the second and third components. To determine if hatchery-raised specimens exhibited allometric growth patterns similar to those observed in wild populations (Bailey and Cross, 1954; Mayden and Kuhajda, 1996), sheared principal components were extracted from a covariance matrix containing small (< 250 mm SL) and large (> 250 mm SL) specimens across all ATUs. Because complete or substantial separation between groups of different-size specimens was observed within each ATU (see Results), subsequent morphometric analyses were computed separately on small and large specimens. To summarize meristic variation within and among ATUs, principal components were extracted from a correlation matrix of 13 meristic characters. Because only four meristic characters were size dependent, the effects on separate-size or combined-size analyses was unclear, and therefore PCA analyses were conducted separately by size and for sizes combined. The broken-stick model (Jackson, 1993; Legendre and Legendre, 1998) was used to evaluate the interpretability of the principal components derived from the meristic data in a correlation matrix relative to those based on random data.

Recording a complete suite of data from live specimens of *Scaphirhynchus* for multivariate analyses is not feasible, and the endangered status of *S. albus* dictates that data collection must be accomplished quickly in the field. To address this need, separate PCAs for both meristic and morphometric data were conducted on reduced data sets of characters that were significantly different between ATUs or loaded heavily along the axes that separated ATUs; feasibility of obtaining data in the field for a particular character was also considered. The meristic PC axis and the morphometric sheared PC axis that realized separation between ATUs in multivariate analyses on both the full and reduced data sets were combined into one bivariate plot to maximize separation between ATUs; this technique has been used to differentiate other hybrid fishes from parentals (Bookstein et al., 1985).

Spine characters

Head armature in *Scaphirhynchus* is typically not considered in the suite of mensural character examined, but these data have been shown to be useful in differentiating between taxa (Mayden and Kuhajda, 1996) and are considered here. Spine measurements were from base to tip of spine. If a spine was bifurcate or represented by more than one spine, the longest spine was measured. Both left and right spine lengths were measured for pre-orbital, parietal, post-temporal, and tabular spines. Condition of the spines was noted as either absent, present but completely fused and forming a ridge, present and partially fused into a ridge, or present and completely exposed.

Other potential characters

Many qualitative characters were recorded from the hatchery-raised specimens to assess differences between ATUs. Bailey and Cross (1954) noted that the outer barbel base was even

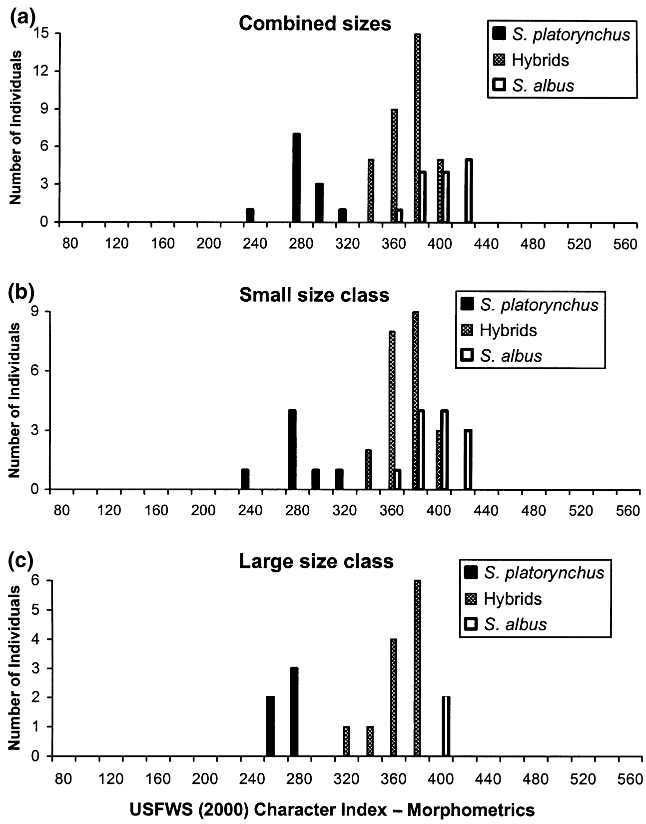

Fig. 3. Character index (U. S. Fish and Wildlife Service, 2000) based on morphometric characters and minimum and maximum values provided with index for (a) all sizes, (b) small (< 250 mm SL), and (c) large (> 250 mm SL) hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

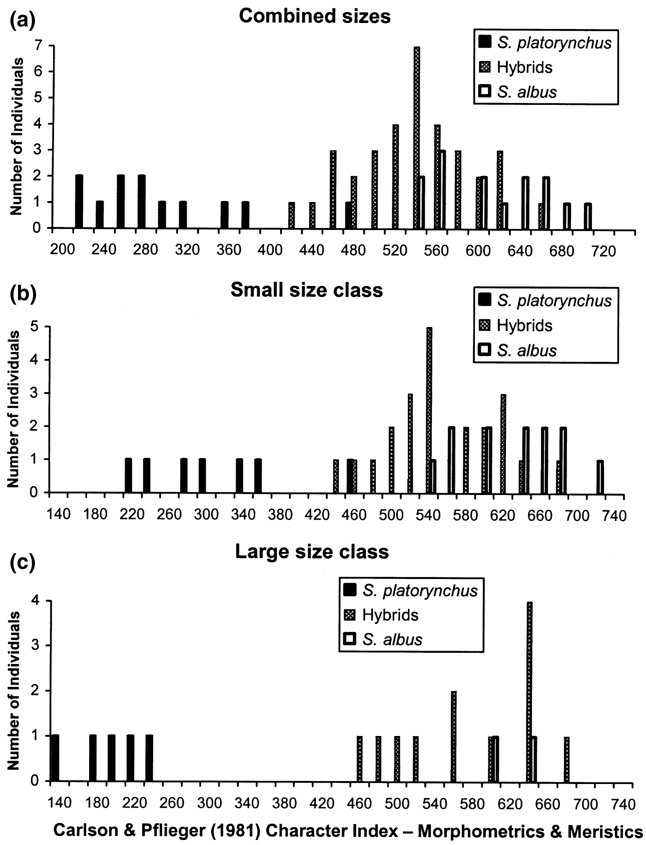

Fig. 4. Character index (Carlson and Pflieger, 1981) based on morphometric and meristic characters for (a) all sizes, (b) small (< 250 mm SL), and (c) large (> 250 mm SL) hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

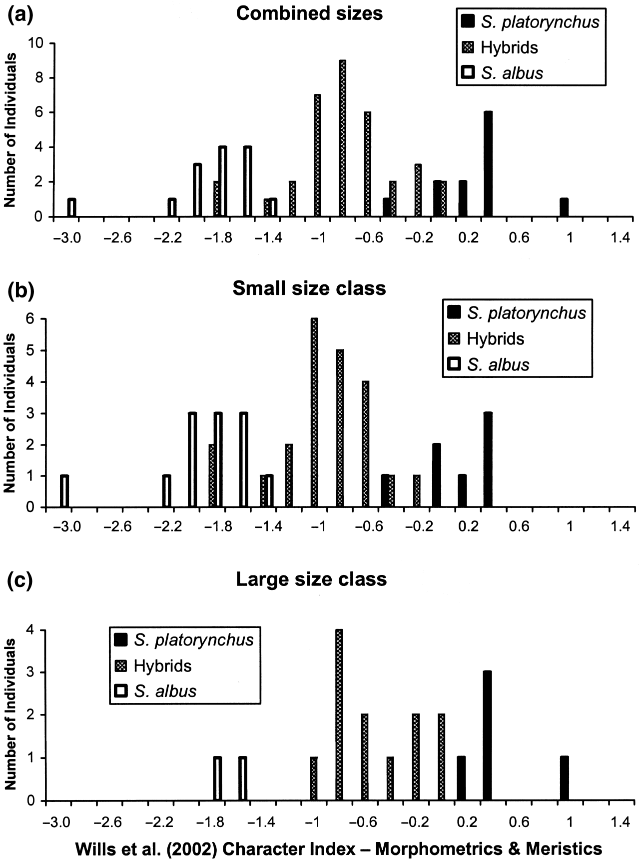

Fig. 5. Character index (Sheehan et al., 1999; Wills et al., 2002) based on morphometric and meristic characters for (a) all sizes, (b) small (<250 mm SL), and (c) large (>250 mm SL) hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

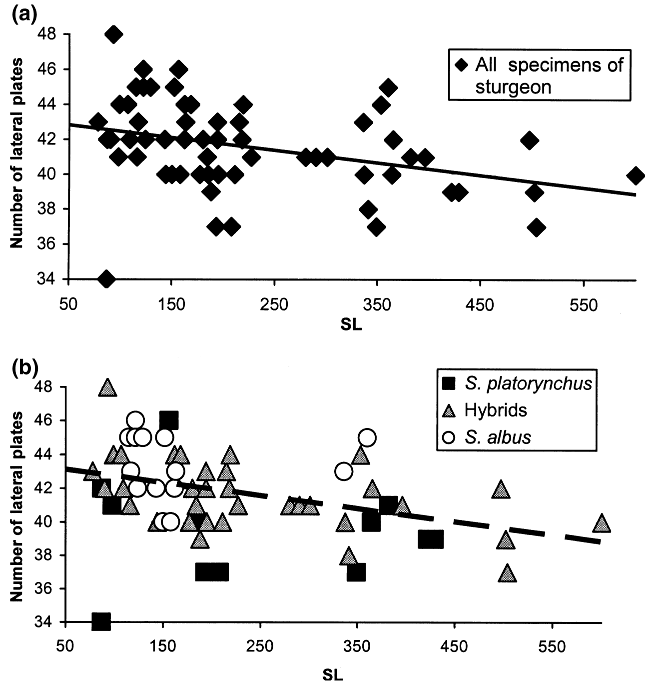

Fig. 6. Relationships of size and lateral plates for hatchery-reared specimens *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*. Trend lines are shown for significant correlations for (a) all *Scaphirhynchus* specimens ($r_s = -0.463$) and (b) hybrids ($r_s = -0.349$)

with or anterior to the base of the inner barbel in *S. platorynchus*, whereas *S. albus* had the outer barbel base posterior to the base of the inner barbels. They also recognized that in adult *S. platorynchus* the belly is mostly scaled and in *S. albus* is mostly naked; this character was not useful for smaller *S. platorynchus*. Bailey and Cross (1954) also noted that the barbel fringe on *S. platorynchus* was better developed relative to *S. albus*. As illustrated by Forbes and Richardson (1905) and noted by Bailey and Cross (1954), *S. platorynchus* have gill rakers that possess more tips and are more fan-like relative to *S. albus*. Other qualitative characters examined included the extent of squamation on the dorsal–lateral and ventral–lateral areas between rows of plates, papillae on the eight lobes of the mouth, the anterior extent of complete armor on the caudal peduncle, spine size on the most posterior ventral–lateral plate with respect to other ventral–lateral plate spines, number of spine present at the posterior-lateral end of the head, presence of a belly ridge, fin pigment pattern (uniform or light-edged), and overall body color.

Results

Character indices

Sample size strongly influences the ability of character indices to discriminate among ATUs and results in inconsistent identification of specimens. Influence of sample size was evident when an index was applied to separate populations of *Scaphirhynchus* from each of three reservoirs in the upper Missouri River (Keenlyne et al., 1994) and contrasted with results in which the three samples were combined. Separate analyses of each population revealed that specimens of *S. albus* and *S. platorynchus* from the headwaters of two upper Missouri reservoirs (Fort Peck and Garrison) could be separated (Fig. 1a,b). In the population from the lower reservoir (Lake Sharpe), three specimens identified in the field as *S. albus* had index values below 300 (Fig. 1c) and were

Table 3
Spearman rank correlation coefficient (r_s) of size (SL) vs meristic variables for specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, *S. albus*, and all analytical taxonomic units (ATUs)

Meristic Character	*S. platorynchus*	*S. platorynchus* × *S. albus*	*S. albus*	All ATUs
Dorsal plates	NS	NS	0.462	NS
Lateral plates	NS	**−0.463**	NS	**−0.410**
Ventral–lateral plates	NS	NS	**−0.450**	NS
Plates between anus and anal fin	0.667	NS	NS	NS
Plates posterior to anal fin	NS	NS	0.112	NS
Pectoral-fin rays	NS	−0.238	**−0.519**	**−0.390**
Pelvic-fin rays	NS	−0.212	−0.484	−0.294
Gill rakers	**0.765**	**0.637**	NS	**0.580**
Tips of gill rakers	**0.939**	**0.874**	NS	**0.824**

Bold indicates P < 0.01; NS refers to not significant.

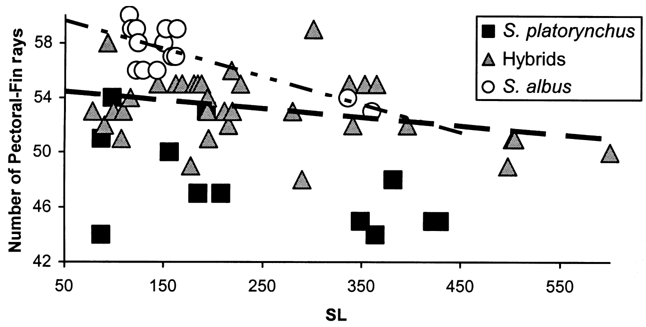

Fig. 7. Relationships of size and pectoral-fin rays for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*. Trend lines are shown for significant correlations for hybrids ($r_s = -0.238$) and *S. albus* ($r_s = -0.519$)

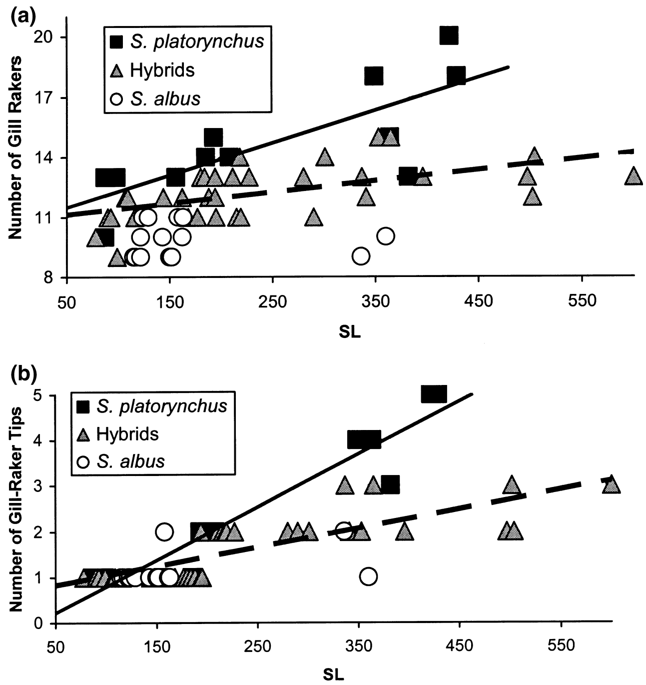

Fig. 8. Relationships of size and gill rakers or gill-raker tips for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*. Trend lines are shown for significant correlations for (a) gill rakers for *S. platorynchus* ($r_s = -0.765$) and hybrids ($r_s = -0.637$) and (b) gill raker tips for *S. platorynchus* ($r_s = -0.939$) and hybrids ($r_s = -0.874$)

considered specimens of questionable purity (Keenlyne et al., 1994). When all three populations in the upper half of the Missouri River (293 specimens) are combined the same three purported hybrid specimens (as well as a fourth *S. albus*) are classified as *S. platorynchus*, indicating that either no potential hybrids are evident or that several specimens of *S. platorynchus* with the same scores as the purported hybrids are also suspect (Fig. 1d).

Specimen size also strongly influences the ability of character indices to discriminate among ATUs. When data from small and combined-size classes of hatchery-reared specimens were evaluated using any of the four character indices, most or all three ATUs had specimens that were misidentified, especially the hybrids and *S. albus*. Large-size class specimens of *S. platorynchus* were typically identified correctly, but large specimens of *S. albus* and hybrids continued to be misidentified.

Using the morphometric formula from Keenlyne et al. (1994), hybrids were indistinguishable from *S. albus* regardless of size class designation, and the specimen with the highest *S. albus*-like score was a hybrid (Fig. 2a–c). Additionally, one small *S. albus* specimen was below the mid-scale score of 300 (Fig. 2a,b). In contrast, hybrids overlapped minimally with *S. platorynchus* in combined and small-size classes (Fig. 2a,b), and were completely separate in the large-size class (Fig. 2c).

Improvement in separating ATUs across all size classes was observed using the USFWS (2000) morphometric index. Hybrids plotted completely separate from *S. platorynchus* in all size comparisons and were distinguished from *S. albus* for the large-size class (Fig. 3a–c). While significant overlap was still prevalent between hybrids and *S. albus* for both combined and small-size classes, several specimens of *S. albus* scored higher than any hybrids (Fig. 3a,b). But hatchery-reared specimens fell well outside the mean score assigned by the USFWS (2000) index for *Scaphirhynchus* species. The 514 reported for *S. albus* was far above the mean score of 414 for the large-size class *S. albus* (Fig. 3c), and differences in mean scores were noted for *S. platorynchus* in all size comparisons between reported (230) vs observed (287–294) scores (Fig. 3a–c).

The Carlson and Pflieger (1981) index, which uses meristic and morphometric data, provided almost complete separation of hybrids from *S. platorynchus* for combined and small-size classes; separation was complete for the large-size class (Fig. 4a–c). But no separation was realized between hybrids and *S. albus* for any size class, although all *S. albus* specimens scored above the mid-scale score of 500 (Fig. 4a–c). For the large-size class, one hybrid specimen actually scored higher than any*S. albus* (Fig. 4c). Because it is difficult to count fin rays on live specimens, the usefulness of the index by Carlson and Pflieger (1981) was examined using only morphometric variables. The moderate separation between the ATUs when meristic data were included was compromised; overlap between hybrids and *S. platorynchus* for both the combined and small-size classes increased. Additionally, complete overlap occurred between hybrids and *S. albus* for all comparisons using only morphometric data, with hybrids having the highest scores, and with many *S. albus* scoring below the middle of the scale.

The morphometric and meristic index developed by Sheehan et al. (1999) and Wills et al. (2002) did not provide separation of hybrids from either *S. albus* or *S. platorynchus* using data from small and combined-size classes (Fig. 5a,b). There was complete separation between ATUs for the large-size class, but several hybrids scored at or below the given mean of −0.86 for *S. albus* (Fig. 5c). Using their morphometric-only index produced similar results for differentiating hybrids from *S. platorynchus* for combined and small-size classes, but overlap of hybrids with *S. albus* was more extensive. Similar results were also obtained for the large-size class, with complete separation between all three ATUs, but several hybrids scored below the mean of −0.69 given for *S. albus*.

Meristic analyses

Meristic data showed mixed relationships of correlation between size and counts. Nine characters were correlated significantly with SL within or across ATUs (Table 3). Four of these characters were correlated significantly within only one ATU (dorsal plates, ventral–lateral plates, plates between anus and anal fin, and plates posterior to anal fin), and pelvic-fin

Table 4
Frequency distribution of four meristic characters for small (<250 mm SL) and large (>250 mm SL) specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

	Lateral plates (specimens <250 mm SL)																	
	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	n	*x*	SD
S. platorynchus	1			2			1	1	1				1			7	39.6	3.95
S. platorynchus × *S. albus*						1	4	3	5	3	5				1	22	42.2	2.02
S. albus							2		3	2		4	1			12	43.2	2.04

	Lateral plates (specimens >250 mm SL)										
	37	38	39	40	41	42	43	44	45	n	*x*	SD
S. platorynchus	1		2	1	1					5	39.2	1.48
S. platorynchus × *S. albus*	1	1	1	2	4	2		1		12	40.5	1.88
S. albus							1		1	2	44.0	1.41

	Pectoral-fin rays (specimens <250 mm SL)																			
	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	n	*x*	SD
S. platorynchus[a]	1			2			1	1		1	1							7	49.4	3.60
S. platorynchus × *S. albus*[b]						1	1	2	2	6	2	7	1		1			22	53.6	1.97
S. albus[c]													3	2	2	4	1	12	57.8	1.40

	Pectoral-fin rays (specimens >250 mm SL)																		
	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	n	*x*	SD
S. platorynchus[a]	1	3			1												5	45.4	1.52
S. platorynchus × *S. albus*[b]					1	1	1	2	2	1		3				1	12	52.5	3.09
S. albus										1	1						2	53.5	0.71

	Gill rakers (specimens <250 mm SL)									
	9	10	11	12	13	14	15	n	*x*	SD
S. platorynchus[a]		1			3	2	1	7	13.1	1.57
S. platorynchus × *S. albus*[a]	1	1	8	6	5	1		22	11.7	1.16
S. albus[b]	5	3	4					12	9.9	0.90

	Gill rakers (specimens >250 mm SL)														
	9	10	11	12	13	14	15	16	17	18	19	20	n	*x*	SD
S. platorynchus[a]					1		1			2		1	5	16.8	2.77
S. platorynchus × *S. albus*[b]			1	2	5	2	2						12	13.2	1.19
S. albus	1	1											2	9.5	0.71

	Gill-raker tips (specimens <250 mm SL)				
	1	2	n	*x*	SD
S. platorynchus	5	2	7	1.3	0.49
S. platorynchus × *S. albus*	16	6	22	1.3	0.46
S. albus	11	1	12	1.1	0.30

	Gill-raker tips (specimens >250 mm SL)							
	1	2	3	4	5	n	*x*	SD
S. platorynchus[a]			1	2	2	5	4.2	0.84
S. platorynchus × *S. albus*[b]		8	4			12	2.3	0.49
S. albus	1	1				2	1.5	0.71

Different letters (a,b,c) indicates character is significantly different between or among ATUs.

rays had no correlation with $P < 0.01$; therefore, all sizes were analyzed together for these characters.

The remaining four characters were correlated significantly with SL at $P < 0.01$ within at least one ATU and across ATUs; they were therefore divided into size classes for subsequent comparisons. Number of lateral plates was significantly and negatively correlated with size for all ATUs combined (Fig. 6a), which can be attributed to hybrids, the only ATU to show a significant correlation (or even a trend) with SL and lateral plate number (Table 3, Fig. 6b). Because

Table 5
Frequency distribution of nine meristic characters for all size-class specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

	Dorsal plates anterior to dorsal fin								
	13	14	15	16	17	18	n	*x*	SD
S. platorynchus		3	4	4		1	12	15.3	1.15
S. platorynchus × *S. albus*	5	7	12	8	2		34	14.9	1.13
S. albus	1	4	4	4	1		14	15.0	1.11

	Dorsal plates posterior to dorsal fin						
	7	8	9	10	n	*x*	SD
S. platorynchus[a]	3	8	1		12	7.8	0.58
S. platorynchus × *S. albus*[b]	1	16	15	2	34	8.5	0.66
S. albus[c]		1	10	3	14	9.1	0.53

	Lateral plates anterior to dorsal fin										
	21	22	23	24	25	26	27	28	n	*x*	SD
S. platorynchus[a]	1		3	3	3	1		1	12	24.3	1.76
S. platorynchus × *S. albus*[b]			2	5	8	10	7	2	34	25.6	1.30
S. albus			4	2	4	4			14	24.6	1.22

	Ventral–lateral plates							
	8	9	10	11	12	n	*x*	SD
S. platorynchus[a]	2	5	5			12	9.3	0.75
S. platorynchus × *S. albus*	1	12	14	6	1	34	9.8	0.87
S. albus[b]		2	6	5	1	14	10.4	0.84

	Ventral plates between anus and anal fin					
	3	4	5	n	*x*	SD
S. platorynchus	4	8		12	3.7	0.49
S. platorynchus × *S. albus*	3	27	4	34	4.0	0.46
S. albus	1	11	2	14	4.1	0.47

	Ventral plates posterior to anal fin						
	7	8	9	10	n	*x*	SD
S. platorynchus[a]	6	6	1		12	7.5	0.52
S. platorynchus × *S. albus*[a]	19	14			34	7.5	0.56
S. albus[b]		5	7	2	14	8.8	0.70

	Dorsal-fin rays															
	30	31	32	33	34	35	36	37	38	39	40	41	42	n	*x*	SD
S. platorynchus[a]	2	2	2	3	2	1								12	32.3	1.61
S. platorynchus × *S. albus*[b]			1	1	5	7	5	8	4	3				34	36.0	1.77
S. albus[c]						1			2	3	4	2	2	14	39.6	1.83

	Anal-fin rays															
	17	18	19	20	21	22	23	24	25	26	27	28	29	n	*x*	SD
S. platorynchus[a]	1	1	3	4	1	1	1							12	19.8	1.64
S. platorynchus × *S. albus*[b]					1	6	8	9	5	4	1			34	23.8	1.45
S. albus[c]									3	4	3	2	2	14	26.7	1.38

	Pelvic-fin rays																
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	n	*x*	SD
S. platorynchus[a]	1	2	4	2	3										12	27.3	1.30
S. platorynchus × *S. albus*[b]					1	1	5	6	14	7					34	32.5	1.24
S. albus[c]									1	1	2	4	5	1	14	36.0	1.36

Different letters (a,b,c) indicates character is significantly different between or among ATUs.

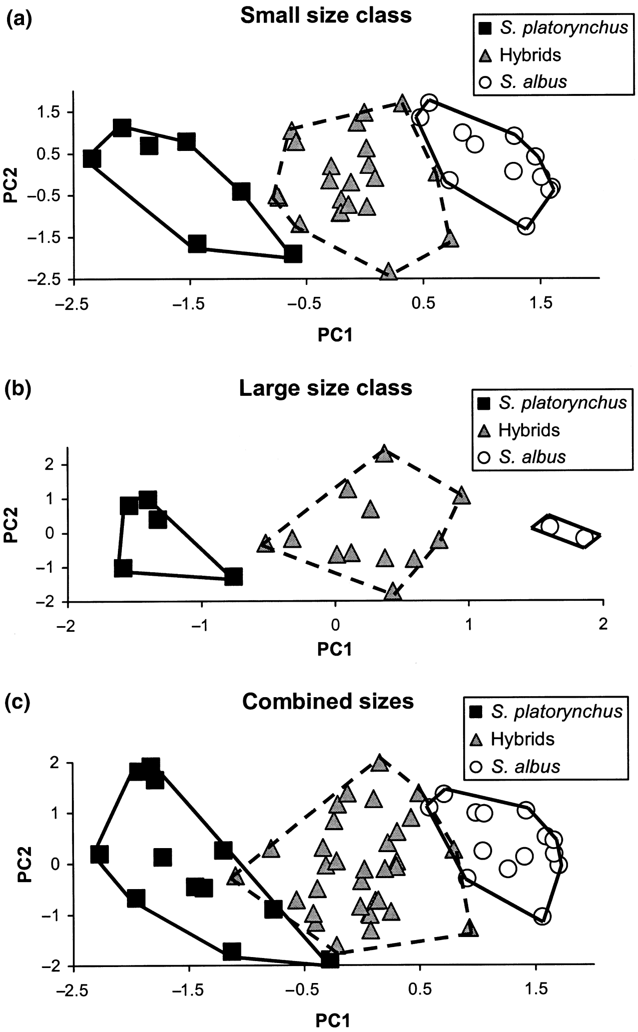

Fig. 9. Principal components analysis of 13 meristic characters for (a) small (< 250 mm SL), (b) large (> 250 mm SL), and (c) all sizes of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

Table 6
Character loadings for principal components analysis of 13 meristic characters for combined small (< 250 mm SL) and large (> 250 mm SL) size classes of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

Meristic character	Loading PC1	Loading PC2
Dorsal plates anterior to dorsal fin	−0.11767	−0.03675
Dorsal plates posterior to dorsal fin	**0.68668**	0.37585
Lateral plates	**0.69959**	−0.38678
Lateral plates anterior to dorsal-fin origin	0.24570	−0.57032
Ventral–lateral plates	0.48337	0.45491
Ventral plates between anus and anal fin	0.20261	0.57884
Ventral plates posterior to anal fin	0.58220	0.29879
Dorsal-fin rays	**0.88902**	0.07202
Anal-fin rays	**0.88385**	0.06187
Pectoral-fin rays	**0.81199**	−0.03786
Pelvic-fin rays	**0.87853**	0.13651
Gill rakers	**−0.75404**	0.34676
Gill raker tips	**−0.59251**	0.52190

See Fig. 9c for graphic representation.
Bold denotes highest positive or negative loadings on PC1 axis.

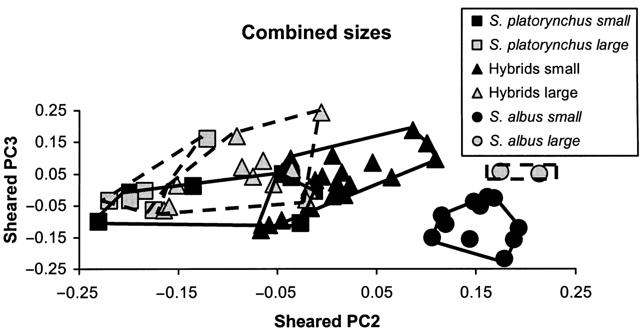

Fig. 10. Sheared principal components analysis of 51 morphometric characters for small (< 250 mm SL) and large (> 250 mm SL) hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

SL and number of lateral plates anterior to the dorsal fin were not correlated, the higher number of plates for smaller specimens was attributable to keeled lateral plates extending further onto the caudal peduncle. A significant decrease in pectoral fin-ray number with increased size occurred across all ATUs and within hybrids and *S. albus*; there was also a similar trend for *S. platorynchus* (Fig. 7). This is likely artifact due to the difficulty in counting the small rays on larger specimens because of thicker fin tissue. The number of gill rakers and gill-raker tips increased significantly with size across ATUs and within *S. platorynchus* and hybrids, but not within *S. albus* (Fig. 8a,b).

Several meristic variables differed significantly among ATUs, but differences were related to size. All three ATUs differed significantly in mean pectoral-fin rays for the small-size class, and no overlap existed between *S. platorynchus* and *S. albus* (Table 4). Only one specimen of small *S. platorynchus* had as few gill rakers as *S. albus*, and the later had significantly fewer gill rakers than the other ATUs. In contrast, all small *Scaphirhynchus* had similar numbers of gill-raker tips (Table 4). There were no significant differences in the means among large *S. albus* and the other ATUs for any of these four meristic characters, but no overlap existed between *S. albus* and *S. platorynchus*. Mean number of pectoral-fin rays, gill rakers, and gill-raker tips were significantly different between *S. platorynchus* and hybrids (Table 4).

Four of the nine meristic characters where all sizes were pooled differed significantly between all three ATUs. These included the mean number of dorsal plates posterior to dorsal fin and mean number of dorsal, anal, and pelvic-fin rays. No overlap between *S. albus* and *S. platorynchus* occurred for number of anal and pelvic-fin rays, and only one specimen overlapped for dorsal-fin ray counts (Table 5). *S. albus* was significantly different from the other ATUs for mean number of ventral plates posterior to the anal fin and from *S. platorynchus* for mean ventral–lateral plate count; *S. platorynchus* differed significantly from hybrids for mean number of lateral plates anterior to dorsal fin (Table 5).

All ATUs were completely separated in meristic space in separate PCA ordinations of small and large specimens and were almost completely separated when sizes were ordinated together (Fig. 9a–c). Eigenvalues exceeded those produced by the broken-stick model in all analyses, supporting the interpretation of the PCA plots. PC1 accounted for 43 to 44 per cent of the variation in all analyses. Univariate analyses provide support for the PCA results. Most variables loading

Table 7
Proportional measurements for small (< 250 mm SL) and large (> 250 mm SL) specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

	S. platorynchus							
	< 250 mm SL (n = 7)				> 250 mm SL (n = 5)			
	Min.	Max.	*x*	CV	Min.	Max.	*x*	CV
Standard length	87	208	144.9	36.7	349	429	389.2	9.1
Snout to caudal fork length	1092	1115	1098	1	1066	1092	1081	11
Snout to dorsal-fin origin	686	710	693	1	692	711	703	1
Snout to pelvic-fin insertion	558	588	572	2	560	576	568	1
Snout to pectoral-fin insertion	295	353	315	6	277	302	292	4
Head length	282	332	305	6	273	291	281	2
Snout to anterior edge subopercle	202	236	219	6	193	208	201	3
Snout to tip of spine at head end	224	263	241	6	206	221	214	3
Snout to anterior edge of orbit	170	193	182	5	163	178	172	3
Snout to anterior edge anterior nostril	133	161	148	7	136	150	144	4
Snout to occiput	236	277	256	6	235	254	244	3
Pectoral-fin to pelvic-fin insertion	244	289	266	7	263	298	286	5
Pectoral-fin length	112	140	125	8	119	137	125	6
Pectoral-fin insertion to occiput	95	114	104	7	95	103	99	3
Body depth at pectoral-fin insertion	86	94	90	3	82	88	85	3
Head depth just anterior to parietal ridge	56	64	60	5	56	65	61	5
Head depth at anterior edge of anterior nostril	39	41	40	2	37	41	39	4
Pelvic-fin length	91	105	97	4	91	99	94	3
Pelvic-fin insertion to anal-fin origin	152	174	159	4	168	184	176	4
Pelvic-fin insertion to dorsal-fin origin	116	139	127	6	142	160	148	5
Dorsal-fin length	79	87	82	3	74	79	77	3
Dorsal-fin base	60	77	69	9	60	66	63	4
Anal-fin to dorsal-fin origin	66	80	71	6	74	81	77	4
Anal-fin origin to last keeled lateral plate	245	282	270	5	249	266	253	3
Caudal peduncle length	206	247	231	7	210	230	218	3
Anal-fin length	85	97	90	4	86	94	89	3
Anal-fin base	35	54	41	15	36	42	39	6
Caudal peduncle depth	15	19	17	8	15	17	16	6
Caudal peduncle width	17	25	20	12	20	25	22	10
Tenth lateral plate height	22	38	32	19	41	46	43	6
Fifth dorsal plate and spine length	17	26	22	14	15	25	21	17
Fifth dorsal plate length	15	22	18	14	15	24	21	16
Fifth dorsal plate and spine height	23	34	30	13	26	30	27	6
Interorbital width	79	84	81	2	75	78	77	2
Orbit length	17	23	19	11	13	15	14	7
Posterior nostril width	30	41	34	11	25	31	28	8
Anterior nostril width	10	15	13	16	8	13	11	14
Pectoral girdle width	110	130	121	6	110	119	115	3
Anterior mouth to pectoral-fin insertion	142	166	150	6	130	146	139	4
Anterior mouth to snout	156	187	171	6	159	172	165	3
Anterior mouth to base of inner barbel	53	77	64	13	54	57	56	3
Anterior mouth to base of outer barbel	73	98	85	10	73	77	75	2
Anterior mouth to head edge at outer barbel	86	108	95	9	82	84	83	1
Snout to base of inner barbel	98	118	107	7	104	116	109	5
Snout to base of outer barbel	106	122	114	6	112	121	115	3
Snout to head edge at anterior mouth	177	198	186	5	172	184	178	3
Outer barbel length	50	74	62	14	64	73	67	5
Inner barbel length	39	58	47	14	47	55	51	5
Head width at outer barbel	118	134	128	5	104	123	114	6
Head width at anterior edge of mouth	144	176	158	8	130	142	136	3
Head width at tip of spine at head end	130	176	154	11	124	134	129	3
Head width at widest point	148	179	163	7	130	143	137	4
Mouth width	65	79	72	7	62	65	64	2

	S. platorynchus × *S. albus*							
	< 250 mm SL (n = 22)				> 250 mm SL (n = 12)			
Standard length	78	227	162.2	30.1	280	600	397.2	26.0
Snout to caudal fork length	1071	1098	1086	1	1068	1104	1081	1
Snout to dorsal-fin origin	679	719	695	1	686	728	667	2
Snout to pelvic-fin insertion	533	615	573	3	554	615	545	2
Snout to pectoral-fin insertion	296	353	322	5	293	353	295	2
Head length	298	341	317	4	292	341	294	3
Snout to anterior edge subopercle	209	244	223	4	200	244	206	4
Snout to tip of spine at head end	219	267	241	5	210	267	219	4

Table 7
(Continued)

	S. platorynchus × *S. albus*							
	< 250 mm SL (n = 22)				> 250 mm SL (n = 12)			
Snout to anterior edge of orbit	176	204	188	4	174	204	176	4
Snout to anterior edge anterior nostril	145	169	156	3	145	169	147	5
Snout to occiput	248	291	267	4	245	291	249	4
Pectoral-fin to pelvic-fin insertion	244	292	260	5	249	300	259	6
Pectoral-fin length	111	141	124	6	111	141	117	5
Pectoral-fin insertion to occiput	93	115	102	6	93	115	96	4
Body depth at pectoral-fin insertion	79	104	89	8	79	104	83	7
Head depth just anterior to parietal ridge	57	77	66	8	58	77	62	5
Head depth at anterior edge of anterior nostril	38	47	41	6	38	47	38	4
Pelvic-fin length	89	112	98	6	83	112	90	6
Pelvic-fin insertion to anal-fin origin	146	172	162	5	160	183	161	4
Pelvic-fin insertion to dorsal-fin origin	114	134	126	4	128	152	128	5
Dorsal-fin length	76	97	84	6	78	97	78	3
Dorsal-fin base	59	81	71	7	63	81	69	7
Anal-fin to dorsal-fin origin	68	85	76	6	78	88	77	4
Anal-fin origin to last keeled lateral plate	246	282	264	3	230	282	247	5
Caudal peduncle length	204	239	224	4	187	239	208	7
Anal-fin length	78	97	86	6	77	97	81	4
Anal-fin base	34	52	42	11	36	52	40	8
Caudal peduncle depth	14	19	16	8	14	19	15	4
Caudal peduncle width	15	23	19	10	20	24	20	6
Tenth lateral plate height	20	41	31	18	36	44	34	6
Fifth dorsal plate and spine length	15	27	20	15	15	27	19	15
Fifth dorsal plate length	13	24	18	17	14	26	18	17
Fifth dorsal plate and spine height	24	32	29	7	23	32	26	8
Interorbital width	72	88	81	5	74	88	75	3
Orbit length	13	22	17	11	11	22	14	14
Posterior nostril width	28	46	34	14	25	46	29	9
Anterior nostril width	9	15	12	13	10	15	11	11
Pectoral girdle width	101	134	121	7	106	134	113	6
Anterior mouth to pectoral-fin insertion	139	174	153	7	141	174	142	3
Anterior mouth to snout	166	190	177	4	161	190	166	5
Anterior mouth to base of inner barbel	49	69	58	7	45	69	52	8
Anterior mouth to base of outer barbel	66	85	75	6	60	85	67	6
Anterior mouth to head edge at outer barbel	81	98	88	5	67	98	77	7
Snout to base of inner barbel	113	132	123	4	118	135	119	4
Snout to base of outer barbel	126	146	136	4	130	148	131	5
Snout to head edge at anterior mouth	175	206	193	4	177	206	180	4
Outer barbel length	55	75	64	8	60	77	63	7
Inner barbel length	31	51	40	12	40	51	40	7
Head width at outer barbel	127	156	139	6	109	156	123	7
Head width at anterior edge of mouth	146	182	160	6	122	182	140	7
Head width at tip of spine at head end	136	178	151	8	116	178	133	9
Head width at widest point	145	186	163	7	123	186	142	7
Mouth width	69	102	78	10	63	102	70	5
	S. albus							
	< 250 mm SL (n = 12)				> 250 mm SL (n = 2)			
Standard length	115	163	138.1	13.3	336	360	348.0	4.9
Snout to caudal fork length	1092	1115	1105	1	1083	1083	1083	0
Snout to dorsal-fin origin	657	681	669	1	679	683	681	0
Snout to pelvic-fin insertion	543	572	555	1	560	561	560	0
Snout to pectoral-fin insertion	285	311	299	3	280	286	283	2
Head length	294	321	308	3	282	283	283	0
Snout to anterior edge subopercle	196	225	210	4	200	202	201	1
Snout to tip of spine at head end	212	241	226	4	205	211	208	2
Snout to anterior edge of orbit	164	185	175	3	172	172	172	0
Snout to anterior edge anterior nostril	135	153	144	4	142	143	143	1
Snout to occiput	243	267	256	3	244	253	249	3
Pectoral-fin to pelvic-fin insertion	250	276	260	3	283	301	292	4
Pectoral-fin length	118	131	126	3	118	121	119	1
Pectoral-fin insertion to occiput	95	110	101	4	91	94	93	2
Body depth at pectoral-fin insertion	88	95	91	3	88	90	89	2
Head depth just anterior to parietal ridge	61	78	72	7	61	64	63	4
Head depth at anterior edge of anterior nostril	39	42	40	3	37	40	38	5
Pelvic-fin length	101	110	105	2	91	99	95	6
Pelvic-fin insertion to anal-fin origin	145	172	164	5	167	174	170	3

Table 7
(Continued)

	S. albus							
	< 250 mm SL (n = 12)				> 250 mm SL (n = 2)			
Pelvic-fin insertion to dorsal-fin origin	120	135	125	3	141	144	142	1
Dorsal-fin length	81	88	84	2	82	83	83	0
Dorsal-fin base	71	83	77	5	73	78	75	5
Anal-fin to dorsal-fin origin	72	90	82	6	81	82	82	1
Anal-fin origin to last keeled lateral plate	263	299	280	4	262	272	267	3
Caudal peduncle length	220	254	240	5	222	230	226	2
Anal-fin length	85	97	90	5	83	84	83	1
Anal-fin base	34	44	40	8	37	41	39	8
Caudal peduncle depth	15	18	16	6	14	15	14	2
Caudal peduncle width	19	23	21	7	18	20	19	9
Tenth lateral plate height	28	35	31	8	37	44	41	13
Fifth dorsal plate and spine length	16	24	21	11	16	16	16	3
Fifth dorsal plate length	13	21	17	12	16	16	16	1
Fifth dorsal plate and spine height	25	29	27	6	27	27	27	1
Interorbital width	74	109	82	11	73	73	73	0
Orbit length	14	17	16	5	13	13	13	1
Posterior nostril width	32	44	37	9	34	35	34	1
Anterior nostril width	10	15	12	11	14	15	14	1
Pectoral girdle width	120	132	126	3	114	115	115	1
Anterior mouth to pectoral-fin insertion	144	161	152	4	146	148	147	1
Anterior mouth to snout	149	239	165	15	151	157	154	3
Anterior mouth to base of inner barbel	45	54	49	7	36	39	38	6
Anterior mouth to base of outer barbel	62	73	68	4	54	55	55	0
Anterior mouth to head edge at outer barbel	78	89	83	4	63	67	65	4
Snout to base of inner barbel	103	123	113	5	121	122	121	1
Snout to base of outer barbel	122	140	129	5	130	132	131	1
Snout to head edge at anterior mouth	159	188	173	4	170	171	171	0
Outer barbel length	53	65	59	6	59	62	60	3
Inner barbel length	27	37	33	10	33	34	33	2
Head width at outer barbel	129	146	138	4	110	113	111	2
Head width at anterior edge of mouth	152	167	161	2	125	128	127	2
Head width at tip of spine at head end	147	163	156	3	121	123	122	2
Head width at widest point	165	182	170	3	132	132	132	0
Mouth width	79	85	82	2	71	74	72	4

All measurements (except standard length) expressed as thousandths of SL and the coefficient of variation (CV) as a per cent.

high and positively on PC1 in the combined size ordination showed significant differences among ATUs in univariate analyses (i.e. all fin-ray counts and number of dorsal plates posterior to dorsal fin), as did variables with large negative loadings (number of gill rakers and gill-raker tips) (Table 6).

Known *Scaphirhynchus* hybrids were meristically intermediate between the parental species. Mean counts for hybrids were intermediate between *S. platorynchus* and *S. albus* in 13 of 17 univariate comparisons. Similarly, modal counts for hybrids were intermediate between parental species in nine of 17 univariate comparisons, and modes of four other variables were shared between all three ATUs (Tables 4 and 5). The range in meristic values for hybrid specimens overlapped the range of both parental species in all but one comparison (gill rakers of large specimens vs *S. albus*), yet there were still several significant differences. The intermediacy of hybrids in univariate analyses is further supported by PCAs of both size classes and combined sizes (Fig. 9a–c).

Hybrids did not demonstrate more meristic variability than their parental species. Hybrids had the highest standard deviation for only four meristic comparisons, whereas *S. platorynchus* had the highest score for nine comparisons (Tables 4 and 5). Because of the similar variability of these meristic characters across all ATUs, hybrids were readily distinguishable from their parental species in the PCA plots (Fig. 9a–c).

Morphometric analyses

Hatchery-raised specimens exhibited clear allometric growth patterns in all ATUs. Complete separation between size classes of *S. albus* was realized in sheared PCA, and substantial separation occurred between different sizes of *S. platorynchus* and hybrids (Fig. 10). Because of allometric growth, small and large-size classes were analyzed separately in subsequent morphometric analyses.

Pairwise comparisons between proportional measurements of ATUs (Table 7) for small and for large-size classes revealed numerous significant differences within each size class (Tables 8 and 9). Both Kruskal–Wallis and ANCOVA analyses within the small-size class revealed that head depth anterior to parietal ridge, anterior edge of mouth to base of outer barbel, and inner barbel length were significant across pairwise comparisons of all ATUs (Table 8). Of the measurements not examined with an ANCOVA, snout to base of outer barbel was significantly different across all pairwise comparisons using the Kruskal–Wallis analysis (Table 8). The only proportional measurements between ATUs that did not have ranges overlapping within the small-size class were snout to dorsal-fin origin and inner barbel length for *S. platorynchus*/*S. albus* and snout to base of outer barbel for the *S. platorynchus*/hybrid comparison (Table 7). No significant differences were detected between large specimens of *S. albus* and the other two ATUs using the ranked sum test (Kruskal–Wallis) (Table 9). Within

Table 8
Statistically significant pairwise comparisons of morphometric characters of small (< 250 mm SL) *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus* using SL as denominator (Kruskal–Wallis) or covariate (ANCOVA)

S. platorynchus/*S. albus*	*S. platorynchus*/hybrids	*S. albus*/hybrids
Kruskal–Wallis/ANCOVA	Kruskal–Wallis/ANCOVA	Kruskal–Wallis/ANCOVA
Snout to dorsal-fin origin	**Head depth anterior to parietal ridge**	Snout to dorsal-fin origin
Snout to pelvic-fin insertion	**Anterior mouth to base outer barbel***	Snout to pelvic-fin insertion
Head depth anterior to parietal ridge	**Inner barbel length***	Snout to pectoral-fin insertion*
Pelvic-fin length	Head width at outer barbel*	Snout to anterior edge subopercle
Dorsal-fin base*		Snout to tip of spine at head end*
Anal-fin to dorsal-fin origin*	Kruskal–Wallis	Snout to anterior edge of orbit
Orbit length	Snout to base of inner barbel*	Snout to anterior edge anterior nostril
Anterior mouth to base of inner barbel*	**Snout to base of outer barbel***	Snout to occiput
Anterior mouth to base of outer barbel*		**Head depth anterior to parietal ridge**
Anterior mouth to head edge outer barbel	ANCOVA	Pelvic-fin length
Snout to head edge at anterior mouth	Snout to pectoral-fin insertion*	Dorsal-fin base
Inner barbel length*	Head length*	Anal-fin to dorsal-fin origin
Head width at outer barbel	Snout to anterior edge subopercle*	Anal-fin origin to last keeled lateral plate
Mouth width*	Snout to anterior edge of orbit*	Caudal peduncle length
	Snout to anterior edge anterior nostril*	Anterior mouth to base of inner barbel*
Kruskal–Wallis	Snout to occiput*	**Anterior mouth to base of outer barbel***
Snout to base of outer barbel	Anal-fin to dorsal-fin origin*	Anterior mouth to head edge outer barbel*
	Orbit length	Snout to head edge at anterior mouth*
ANCOVA	Anterior mouth to base of inner barbel	**Inner barbel length***
Snout to pectoral-fin insertion	Anterior mouth to head edge outer barbel*	Head width at widest point
Snout to tip of spine at head end	Snout to head edge at anterior mouth*	Mouth width
Head width at anterior mouth*	Head width at anterior mouth	
Head width at widest point	Mouth width*	
		Kruskal–Wallis
		Anal-fin length
		Anterior mouth to snout
		Snout to base of inner barbel
		Snout to base of outer barbel
		ANCOVA
		Head length*
		Orbit length

Characters are grouped as significant in both analyses or only in a single analysis. Characters significant across all pairwise comparisons in bold. Characters significant here and in same pairwise comparison for large (> 250 mm SL) *Scaphirhynchus* (Table 9) denoted with asterisk.

Table 9
Statistically significant pairwise comparisons of morphometric characters of large (> 250 mm SL) *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus* using SL as denominator (Kruskal–Wallis) or covariate (ANCOVA)

S. platorynchus/*S. albus*	*S. platorynchus*/hybrids	*S. albus*/hybrids
ANCOVA	Kruskal–Wallis/ANCOVA	ANCOVA
Dorsal-fin length	Head length*	Snout to pectoral-fin insertion*
Dorsal-fin base*	Snout to anterior edge of orbit*	Head length*
Anal-fin to dorsal-fin origin*	Snout to anterior edge anterior nostril*	Snout to tip of spine at head end*
Posterior nostril width	Snout to occiput*	Interorbital width
Anterior nostril width	Dorsal-fin base	Posterior nostril width
Anterior mouth to base of inner barbel*	Anal-fin to dorsal-fin origin*	Anterior mouth to snout
Anterior mouth to base of outer barbel*	Tenth lateral plate height	Anterior mouth to base of inner barbel*
Anterior mouth to head edge outer barbel	**Anterior mouth to base outer barbel***	**Anterior mouth to base outer barbel***
Snout to base of outer barbel	Snout to base of inner barbel*	**Anterior mouth to head edge outer barbel***
Inner barbel length*	Snout to base of outer barbel*	Snout to head edge at anterior mouth*
Head width at anterior mouth*	Snout to head edge at anterior mouth*	**Inner barbel length***
Mouth width*	**Inner barbel length***	Head width at outer barbel
	Mouth width*	Head width at anterior mouth
		Head width at tip of spine at head end
	Kruskal–Wallis	
	Anterior mouth to pectoral-fin insertion	
	ANCOVA	
	Snout to pectoral-fin insertion*	
	Snout to anterior edge subopercle*	
	Snout to tip of spine at head end	
	Pelvic-fin insertion to dorsal-fin origin	
	Anal-fin base	
	Anterior mouth to head edge outer barbel*	
	Head width at outer barbel*	

Characters are grouped as significant in both analyses or only in a single analysis. Characters significant across all pairwise comparisons in bold. Characters significant here and in same pairwise comparison for small (< 250 mm SL) *Scaphirhynchus* (Table 8) denoted with asterisk.

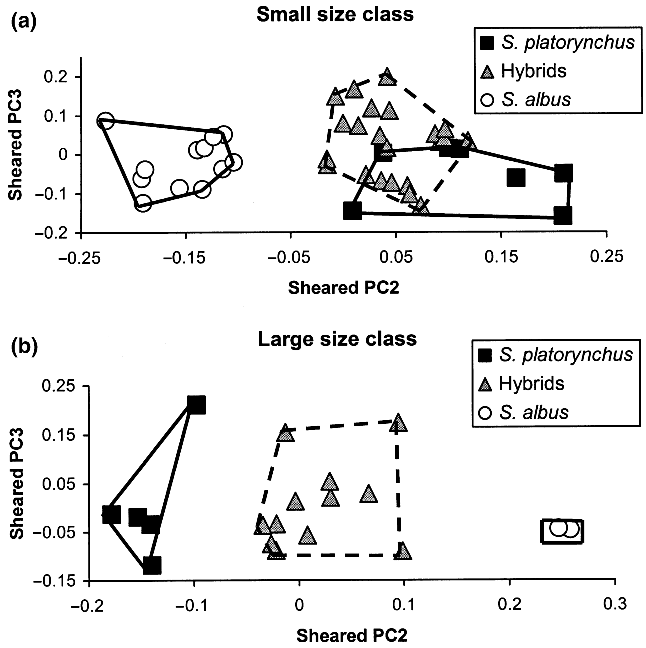

Fig. 11. Sheared principal components analysis of 51 morphometric characters for (a) small (< 250 mm SL) and (b) large (> 250 mm SL) hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

the ANCOVA analysis, variables that were significant between all pairwise comparisons of ATUs were anterior edge of mouth to base of outer barbel, anterior edge of mouth to head edge outer barbel, and inner barbel length (Table 9). Numerous ranges of proportional measurements of large specimens of *S. albus* did not overlap with the other two ATUs. For the *S. platorynchus*/hybrid comparison, ranges for head length and snout to base of inner and to base of outer barbel did not overlap (Table 7).

Several variables were significantly different in the same pairwise comparisons between ATUs for both small and large specimens. Seven characters were significant for both size classes for the *S. platorynchus*/*S. albus* comparison (ANCOVA only), eight characters for the *S. albus*/hybrid comparison (ANCOVA only); the comparison of *S. platorynchus*/hybrids yielded two significant characters for both ratio and covariate analyses, two shared characters within the ranked sum test, and 11 characters for the ANCOVA (Tables 8 and 9).

Sheared PCA of morphometric characters for small-size class specimens showed complete separation of *S. albus* from the other ATUs along PC2 (Fig. 11a). Characters with highest absolute magnitudes on PC2 contrasted orbit length, anterior edge of mouth to base of inner and to base of outer barbel, and inner barbel length (positive loadings) with head depth anterior to parietal ridge, dorsal-fin base, and anal-fin to dorsal-fin origin (negative loadings) (Table 10). Only moderate separation occurred between *S. platorynchus* and hybrids along both PC2 and PC3 (Fig. 11a). PC3 contrasted anterior nostril width and snout to base of outer barbel with tenth lateral plate height, fifth dorsal plate length, and fifth dorsal plate and spine length (Table 10). Complete separation between all three ATUs was realized along the sheared PC2 axis for large specimens (Fig. 11b). This axis contrasted dorsal-fin base, anterior nostril width, and snout to base of outer barbel with fifth dorsal plate length, fifth dorsal plate and spine length, anterior edge of mouth to base of inner and to base of outer barbel, anterior edge of mouth to edge of head at outer barbel base, and inner barbel length (Table 11).

Overall, hybrids showed intermediacy to parental species in morphometry. Means of 17 proportional measurements of small-size class hybrids were intermediate to their parental species, but more hybrid variables (21) had means closer to those of *S. platorynchus* than to *S. albus* (10) (Table 7). This was evident in the sheared PCA for small specimens, where hybrids and *S. platorynchus* overlapped (Fig. 11a). Although similar relationships were present for the large-size class (18 hybrid means intermediate, 17 and 11 means closer to *S. platorynchus* and to *S. albus*, respectively) (Table 7), the sheared PCA shows that hybrids were intermediate in shape relative to the parental species (Fig. 11b).

Morphometric variability of hybrids with respect to the parental species depended on size class. For the small-size class, *S. platorynchus* had 32 characters with the highest CV compared to only 17 characters for hybrids (Table 7). The large-size class hybrids were much more variable, with 36 characters possessing the highest CV compared to only 12 characters for *S. platorynchus* (Table 7). But even with this higher variability, there was complete separation of hybrids from their parental species in the sheared PCA ordination (Fig. 11b).

Combined axes from meristic and morphometric PCAs and reduced dataset

Differentiation between *S. platorynchus*, *S. albus*, and hybrids is maximized by combining PC1 meristic and sheared PC2 morphometric axes into a bivariate plot (Fig. 12a,b). Only slight overlap occurred within the small-size class between *S. platorynchus* and hybrids; otherwise, complete separation between ATUs was realized (Fig. 12a,b).

Characters used in the reduced data set for multivariate analyses were those that were feasible for data collection in the field, were significantly different between ATUs in univariate analyses, and were loaded heavily along axes that separated ATUs in multivariate analyses. Pectoral and pelvic-fin rays were excluded because of the difficulty in counting rudimentary rays. Gill rakers and raker tips were eliminated because of the possibility of serious injury to sturgeon when examining these characters. Characters that met the above criteria included six meristic and 12 morphometric characters (Table 12). Complete separation among ATUs in both size classes is obtained by conducting the ordinations with this reduced data set (Fig. 13a,b). Excluding seven meristic variables from the data set reduced the separation of ATUs along PC1 for both size classes. A slight decrease in separation with the reduced data set also was noted along the sheared PC2 axis for the large-size class (Fig. 13b), but using only the 12 morphometric characters actually increased the separation within the small-size class between *S. platorynchus* and hybrids (Fig. 13a).

Spine characters

Other than the snout spines, all head spines were represented in examined hatchery-reared specimens. Ten specimens across ATUs had bifurcate or trifurcate spines. All of these specimens were < 200 mm SL, suggesting that these spines may fuse as specimens get larger. The degree of spine fusion and SL were significantly and positively correlated for all eight spine characters when all three ATUs were examined together. As

Table 10
Character loadings for sheared principal components analysis (SPC) of 51 morphometric characters for small (< 250 mm SL) *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

	Loading		
	Size	SPC2	SPC3
Morphometric character			
Standard length	0.14685	−0.03531	0.02413
Snout to dorsal-fin origin	0.14594	0.03034	0.03829
Snout to pelvic-fin insertion	0.14225	0.02987	0.05792
Snout to pectoral-fin insertion	0.13044	0.08899	0.06508
Head length	0.13189	0.00747	0.06573
Snout to anterior edge subopercle	0.13203	0.07817	0.10059
Snout to tip of spine at head end	0.13036	0.09462	0.09063
Snout to anterior edge of orbit	0.13795	0.08250	0.08513
Snout to anterior edge anterior nostril	0.13923	0.08856	0.09722
Snout to occiput	0.13035	0.02307	0.06839
Pectoral-fin to pelvic-fin insertion	0.15691	−0.02086	0.04181
Pectoral-fin length	0.13487	−0.05115	0.00931
Pectoral-fin insertion to occiput	0.12896	0.00657	0.00878
Body depth at pectoral-fin insertion	0.12781	−0.05750	0.00834
Head depth just anterior to parietal ridge	0.12948	**−0.25785**	0.03210
Head depth anterior edge of anterior nostril	0.13406	−0.00611	0.06093
Pelvic-fin length	0.13894	−0.16659	−0.00557
Pelvic-fin insertion to anal-fin origin	0.15298	−0.09385	−0.05008
Pelvic-fin insertion to dorsal-fin origin	0.15620	−0.04243	−0.06397
Dorsal-fin length	0.13645	−0.07549	0.02123
Dorsal-fin base	0.13881	**−0.21810**	−0.07535
Anal-fin to dorsal-fin origin	0.15570	**−0.21556**	−0.05775
Anal-fin origin to last keeled lateral plate	0.15438	−0.13563	0.03608
Caudal peduncle length	0.15595	−0.15985	0.02412
Anal-fin length	0.14232	−0.09474	0.00092
Anal-fin base	0.14393	0.11209	0.07874
Caudal peduncle depth	0.12789	0.03001	−0.04176
Caudal peduncle width	0.16341	−0.12006	−0.17369
Tenth lateral plate height	0.20594	−0.10229	**−0.25115**
Fifth dorsal plate and spine length	0.12750	0.07162	**−0.51245**
Fifth dorsal plate length	0.15396	0.09614	**−0.60427**
Fifth dorsal plate and spine height	0.15404	0.01883	−0.04900
Interorbital width	0.13685	−0.02117	0.00214
Orbit length	0.11525	**0.19649**	0.09448
Posterior nostril width	0.11026	−0.13129	0.12503
Anterior nostril width	0.14360	0.10177	**0.19458**
Pectoral girdle width	0.12785	−0.08302	−0.06429
Anterior mouth to pectoral-fin insertion	0.12517	−0.04800	0.01045
Anterior mouth to snout	0.14375	0.12706	0.14220
Anterior mouth to base of inner barbel	0.12665	**0.35915**	0.03098
Anterior mouth to base of outer barbel	0.13007	**0.24667**	−0.09083
Anterior mouth to head edge at outer barbel	0.13139	0.16153	−0.02564
Snout to base of inner barbel	0.15008	0.01768	0.14612
Snout to base of outer barbel	0.14627	−0.05378	**0.17058**
Snout to head edge at anterior mouth	0.13950	0.14272	0.10766
Outer barbel length	0.11791	0.14524	0.06880
Inner barbel length	0.10726	**0.50199**	−0.08805
Head width at outer barbel	0.13307	−0.06753	0.10854
Head width at anterior edge of mouth	0.12382	−0.03111	0.05252
Head width at tip of spine at head end	0.12134	−0.06946	0.01841
Head width at widest point	0.12356	−0.08933	0.02623
Mouth width	0.12300	−0.13626	0.05871

See Fig. 11a for graphic representation.
Bold denotes highest positive or negative loadings on a given axis.

specimens increased in size, the spines tended to become partially or completely fused into a ridge. Pre-orbital and parietal spine fusion was correlated positively with SL for *S. platorynchus* and hybrids, but not for *S. albus* (Fig. 14a,b), whereas only *S. albus* was correlated with SL for post-temporal spine fusion (Fig. 14c). Tabular spine fusion was correlated significantly with SL for all ATUs (Fig. 14d). Because of these relationships, frequency distributions of spine fusion were examined by size class.

All small specimens had parietal and tabular spines present and the degree of fusion between the three ATUs was similar (Table 13). Most *S. platorynchus* and hybrid specimens had pre-orbital spines exposed or only partially fused; in *S. albus* specimens most of these spines were completely fused or missing. Conversely, most specimens of *S. albus* had prominent post-temporal spines, whereas these spines were absent or fused in numerous *S. platorynchus* (Table 13). For the large-size class, all specimens had tabular spines, and only

Table 11
Character loadings for sheared principal components analysis (SPC) of 51 morphometric characters for large (>250 mm SL) *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

	Loading		
	Size	SPC2	SPC3
Morphometric characters			
Standard length	0.13911	0.03306	0.01975
Snout to caudal fork length	0.14037	0.03813	0.01992
Snout to dorsal-fin origin	0.14899	0.01642	0.02662
Snout to pelvic-fin insertion	0.14933	0.03742	0.04096
Snout to pectoral-fin insertion	0.13664	0.01975	0.06624
Head length	0.13404	0.05851	0.08142
Snout to anterior edge subopercle	0.13121	0.04532	0.08919
Snout to tip of spine at head end	0.12667	0.01205	0.10104
Snout to anterior edge of orbit	0.13427	0.05170	0.10061
Snout to anterior edge anterior nostril	0.13508	0.05180	0.13252
Snout to occiput	0.12986	0.06918	0.10141
Pectoral-fin to pelvic-fin insertion	0.15263	0.06506	0.00260
Pectoral-fin length	0.13476	−0.02977	−0.02601
Pectoral-fin insertion to occiput	0.14369	−0.01964	0.08260
Body depth at pectoral-fin insertion	0.16317	0.06152	−0.04727
Head depth just anterior to parietal ridge	0.13261	0.07024	0.05302
Head depth anterior edge of anterior nostril	0.13755	0.02229	0.01744
Pelvic-fin length	0.12655	0.03128	−0.00563
Pelvic-fin insertion to anal-fin origin	0.15241	0.01478	0.04790
Pelvic-fin insertion to dorsal-fin origin	0.15771	−0.02131	−0.05373
Dorsal-fin length	0.13739	0.09911	0.00785
Dorsal-fin base	0.15878	**0.27367**	−0.05981
Anal-fin to dorsal-fin origin	0.15659	0.13037	0.02712
Anal-fin origin to last keeled lateral plate	0.11681	0.06955	−0.04140
Caudal peduncle length	0.10442	0.03276	−0.04963
Anal-fin length	0.13658	−0.05347	0.05266
Anal-fin base	0.17710	0.11795	−0.01582
Caudal peduncle depth	0.14914	−0.04626	0.06465
Caudal peduncle width	0.14691	−0.10806	−0.05122
Tenth lateral plate height	0.15450	−0.08900	0.03431
Fifth dorsal plate and spine length	0.18938	**−0.25858**	−0.50717
Fifth dorsal plate length	0.19572	**−0.25409**	−0.54983
Fifth dorsal plate and spine height	0.15975	0.01242	0.07575
Interorbital width	0.12842	−0.02137	0.02046
Orbit length	0.07786	−0.14560	0.05024
Posterior nostril width	0.09315	0.16647	−0.04424
Anterior nostril width	0.12814	**0.28736**	−0.43968
Pectoral girdle width	0.13429	0.03862	0.03432
Anterior mouth to pectoral-fin insertion	0.14081	0.11770	0.02080
Anterior mouth to snout	0.13335	−0.01458	0.12951
Anterior mouth to base of inner barbel	0.12989	**−0.39393**	0.11299
Anterior mouth to base of outer barbel	0.12505	**−0.31595**	0.13750
Anterior mouth to head edge at outer barbel	0.11086	**−0.24749**	0.09124
Snout to base of inner barbel	0.13627	0.18278	0.10154
Snout to base of outer barbel	0.14163	**0.21726**	0.06886
Snout to head edge at anterior mouth	0.13256	0.01147	0.08081
Outer barbel length	0.14715	0.00113	0.06686
Inner barbel length	0.15439	**−0.33209**	0.20818
Head width at outer barbel	0.11125	−0.00062	−0.02181
Head width at anterior edge of mouth	0.11183	−0.06452	0.01083
Head width at tip of spine at head end	0.10358	−0.05729	−0.02106
Head width at widest point	0.11068	−0.03109	−0.00881
Mouth width	0.12328	0.15045	−0.01093

See Fig. 11b for graphic representation.
Bold denotes highest positive or negative loadings on SPC2 axis.

one or two specimens were missing pre-orbital and parietal spines (Table 14). Post-temporal and parietal spines were poorly developed in all ATUs, and several hybrid specimens lacked post-temporal spines (Table 14).

Proportional spine measurements differed between ATUs in both size classes (Tables 15 and 16). Within the small-size class, *S. albus* had significantly smaller right pre-orbital spines than either *S. platorynchus* or hybrids for both univariate analyses and also possessed a smaller left pre-orbital spine than either ATU for the Kruskal–Wallis test. The left post-temporal spine of *S. albus* was also significantly smaller relative to *S. platorynchus* (Tables 15 and 16). Only the ANCOVA detected any significant differences in spine size within the large-size class. Both post-temporal spines and the right tabular spine were significantly smaller in *S. albus* relative to *S. platorynchus*; in hybrids the right post-temporal spine was

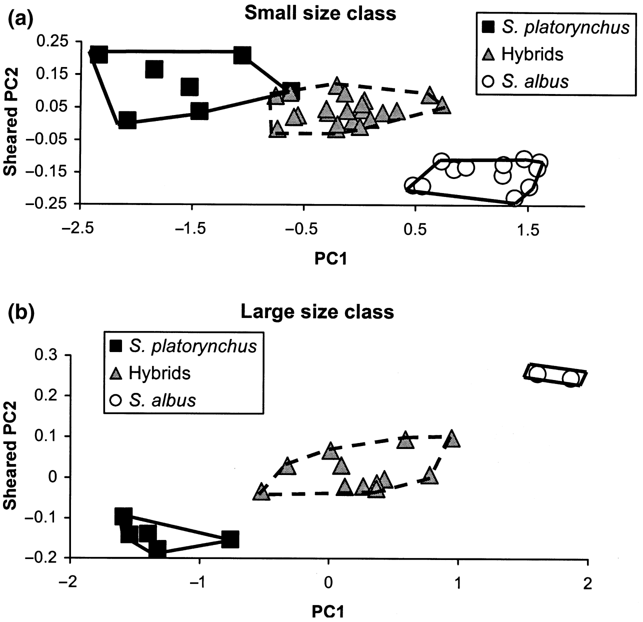

Fig. 12. First principal components axis from PCA of 13 meristic characters and second principal components axis from sheared PCA of 51 morphometric characters for (a) small (< 250 mm SL) and (b) large (> 250 mm SL) hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

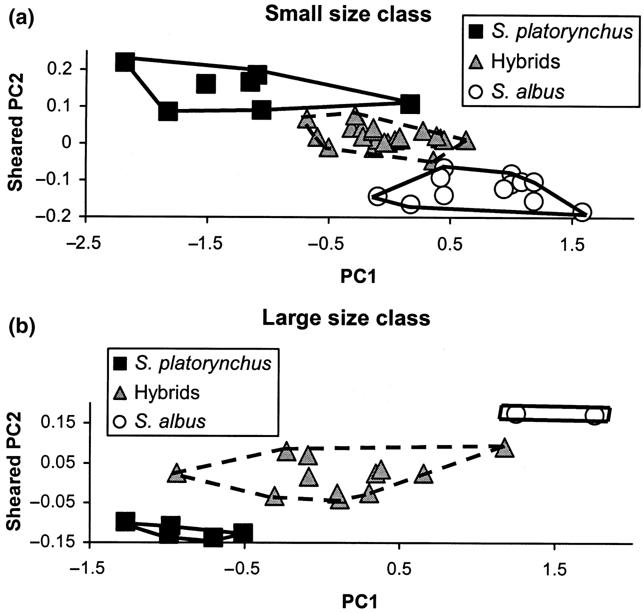

Fig. 13. First principal components axis from PCA of six meristic characters and second principal components axis from sheared PCA of 12 morphometric characters for (a) small (< 250 mm SL) and (b) large (> 250 mm SL) hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

Table 12
Six meristic and 12 morphometric characters used in the reduced data set for PCA analyses of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

Meristic characters	Morphometric characters
Dorsal plates posterior to dorsal fin	**Head depth anterior parietal ridge**
Lateral plates	**Dorsal-fin base**
Lateral plates anterior to dorsal fin	**Anal to dorsal-fin origin**
Ventral plates posterior to anal fin	**Fifth dorsal plate & spine length**
Dorsal-fin rays	Orbit length
Anal-fin rays	Anterior mouth to base inner barbel
	Anterior mouth to base outer barbel
	Anterior mouth to head edge outer barbel
	Snout to base outer barbel
	Inner barbel length
	Head width anterior edge mouth
	Mouth width

Characters in bold have not been used in character indices.

also smaller than that in *S. platorynchus*. Hybrids had significantly larger right parietal spines relative to the other ATUs (Tables 15 and 16).

In most cases, spine size for hybrids was typically intermediate relative to the parental species. For both small- and large-size classes, six of eight spine characters in hybrids showed intermediacy (Table 15).

Additional characters

The outer barbel base was even with or anterior to the base of the inner barbel in *S. platorynchus*, whereas *S. albus* had the outer barbel base posterior to the base of the inner barbels. Some hybrids had outer barbels even with inner barbels, but most, including all > 300 mm SL, had outer barbels posterior to inner barbels (Table 17).

Four of five large-size class specimens of *S. platorynchus* had a belly mostly scaled whereas all large *S. albus* and hybrids had naked bellies. Five of seven small *S. platorynchus* lacked belly squamation, as did all small *S. albus* and hybrids. All specimens of *S. albus* lacked rhomboid scales or small plates on the dorsal–lateral area; only light spicules were present. This contrasts with *S. platorynchus* and hybrids, in which some specimens > 300 mm SL had small, embedded scales as well as the presence of light spicules; other large and all small specimens were similar to *S. albus*. No specimens had any scales present in the ventral–lateral area, but all *S. platorynchus* had spicules across this area, while most *S. albus* and hybrids had reduced spicules or none at all.

The barbel fringe on *S. platorynchus* was better developed relative to *S. albus*. Large specimens of *S. platorynchus* had barbel papillae that were complex and branching, both in the row of papillae on the leading edge of the barbel and the two rows on the posterior-lateral edges. Specimens in the small-size class but > 100 mm SL had mostly simple unbranched papillae on the leading edge, but had branching on the other papillae; specimens < 100 mm SL had only small, simple papillae. This contrasts with *S. albus*, where specimens 140–360 mm SL lacked branching papillae on the leading edge, and smaller specimens down to 115 mm SL had only small, simple papillae. Large specimens of hybrids did not have barbel fringe as complex as *S. platorynchus*, but more so than *S. albus*. Very small hybrids (< 110 mm SL) had practically no papillae on the leading edge of the barbel, and only very small and simple papillae on the posterior-lateral edge. For all ATUs, the fringe was better developed on the distal two-thirds of the barbel.

The papillae on the eight lobes of the mouth followed the same pattern as the papillae on the barbels. All large specimens of *S. platorynchus* and two specimens just below 200 mm SL had numerous long and thick papillae on the lobes of the

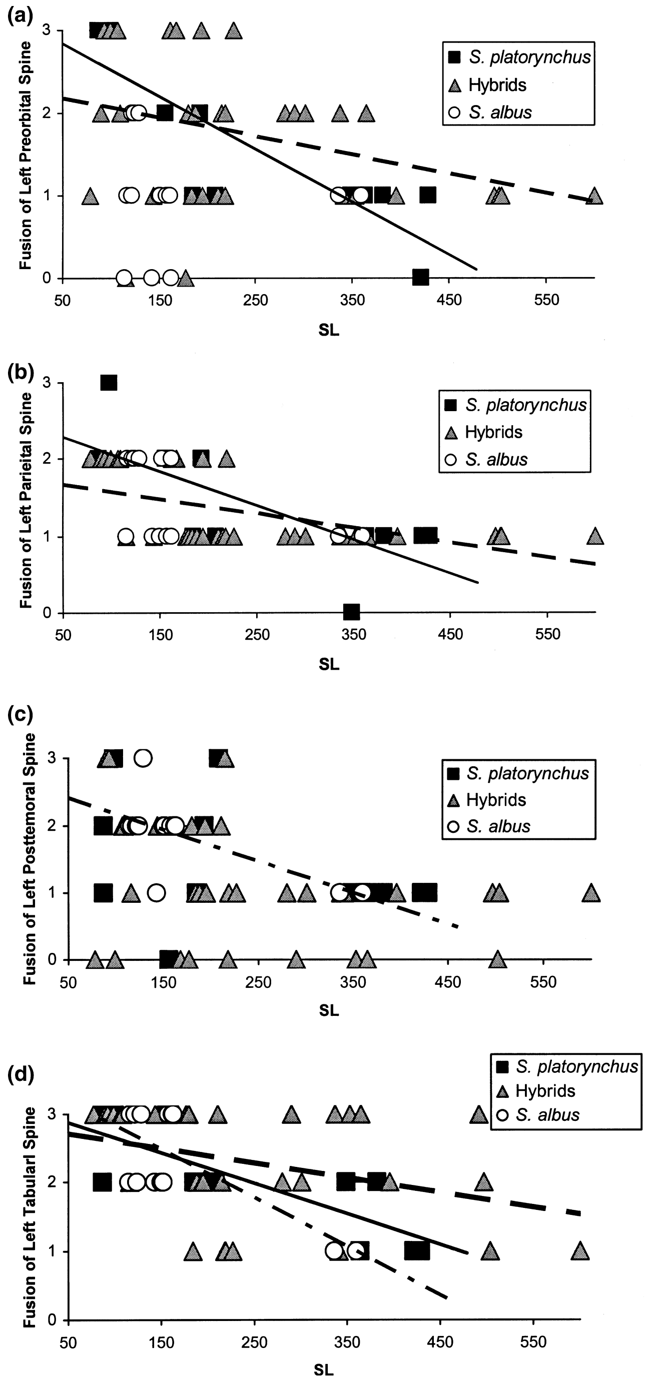

Fig. 14. Relationships of head spine fusion with size for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*. Trend lines are shown for significant correlations of spine fusion of (a) left pre-orbital in *S. platorynchus* ($r_s = -0.872$) and hybrids ($r_s = -0.318$), (b) left parietal in *S. platorynchus* ($r_s = -0.736$) and hybrids ($r_s = -0.648$), (c) left post-temporal in *S. albus* ($r_s = -0.461$), and (d) left tabular in *S. platorynchus* ($r_s = -0.804$), hybrids ($r_s = -0.447$), and *S. albus* ($r_s = -0.333$)

mouth, with many of the papillae branched. Other specimens in the small-size class but above 100 mm SL had papillae slightly shorter and with very few branches. Specimens < 100 mm SL had much smaller, fewer, and simpler papillae. These simple papillae were the only type present in *S. albus*; several specimens had papillae reduced to no more than a few knobs on each lobe. Hybrids possessed mouth papillae very similar to *S. platorynchus*, with several large specimens having complex and large papillae; no specimens had papillae reduced to knobs such as in *S. albus*.

Scaphirhynchus platorynchus had gill rakers that possessed more tips (3–5) and were more fan-like relative to *S. albus* (1–2 tips). It was also noted that the gill rakers in *S. albus* were stiff and remained erect in preserved specimens, while those of *S. platorynchus* were malleable and tended to lie flat against the arch. Hybrids were intermediate for this character. These differences were less apparent in the smallest specimens.

Most specimens of *S. albus* and *S. platorynchus* had completely armored caudal peduncles extended anteriad to just anterior to the anal-fin origin. Caudal peduncle armor was not as extensive in three and one specimens of *S. albus* and *S. platorynchus*, respectively, and five specimens of *S. platorynchus* had armor extending further forward to just posterior to the dorsal-fin origin. Just over half of the hybrid specimens exhibited typical caudal peduncle armature of both parental species. Of the remaining specimens, eight (< 165 mm SL) had reduced armature, while seven (> 175 mm SL) had more extensive caudal peduncle armor.

Numerous specimens in all ATUs had two rather than one spine present at the posterior-lateral end of the head (Table 18). All of these specimens, except one *S. platorynchus*, were in the small-size class. These double spines may fuse as the individual increases in size. The size of the spine on the most posterior ventral–lateral plate with respect to other ventral–lateral plate spines also appears to be related to the size of the specimen. Almost all specimens in the small-size class had this spine equal or larger in size; in two hybrids, this spine was slightly smaller. In nearly half (nine) of the specimens in the large-size class, this spine was worn off. Large specimens with spines were at best only slightly larger relative to other ventral–lateral spines.

All but one specimen in the small-size class possessed a prominent ridge or flap of skin along the midline of the belly. This ridge was not present in any large-size class specimens nor in a 208 mm SL *S. platorynchus*. Size also played a factor in fin color. All but one small specimen had uniform coloration of all fins, whereas large specimens of all *S. albus* and *S. platorynchus*, and most hybrids, had a light edge along both paired and unpaired fins. However, no consistent differences in body color of preserved specimens were apparent between the size classes or ATUs.

Discussion

This study was the first to use a data set obtained from hatchery-reared specimens of *Scaphirhynchus* representing 'known' individuals of *S. platorynchus*, *S. albus*, and their hybrids. The use of specimens bred in a controlled environment had numerous advantages over using wild-caught specimens in addressing our objectives, especially for hybrids, but there were several shortcomings using hatchery-reared specimens. The sample size was small for some ATUs, especially for the large-size class. Additionally, all brood stock came from the extreme upper Missouri River, thus the geographic coverage was limited. Since there was no method to establish 'pure' *S. albus* and *S. platorynchus* at the time these specimens were produced, it was only assumed that the stocks were pure because no hybrids were known from this part of the drainage. Only a one-way cross (two male *S. albus* × one female *S. platorynchus*) was made. If a female *S. albus* had been crossed with a male *S. platorynchus*, the resulting hybrids may have possessed different character states. All specimens were

Table 13
Frequency distribution of spines and spine fusion on head region for small (< 250 mm SL) specimens of *Scaphirhynchus platorynchus* (n = 7), *S. platorynchus* × *Scaphirhynchus albus* (n = 22), and *S. albus* (n = 12)

	0	1	2	3	0	1	2	3
	Left pre-orbital spine				Right pre-orbital spine			
S. platorynchus		2	2	3		1	3	3
S. platorynchus × *S. albus*	2	6	6	8	2	5	5	10
S. albus	3	6	3		1	7	4	
	Left parietal spine				Right parietal spine			
S. platorynchus		2	4	1		2	5	
S. platorynchus × *S. albus*		12	10			11	10	1
S. albus		5	7			7	5	
	Left post-temporal spine				Right post-temporal spine			
S. platorynchus	1	2	2	2	2	3	2	
S. platorynchus × *S. albus*	5	6	8	3	2	9	5	6
S. albus		1	10	1		1	7	4
	Left tabular spine				Right tabular spine			
S. platorynchus			4	3			3	4
S. platorynchus × *S. albus*		4	6	12		1	9	12
S. albus			5	7			2	10

For all head spines: 0, spine absent; 1, present but completely fused, forming a ridge; 2, present and partially fused into ridge; 3, present and exposed.

Table 14
Frequency distribution of spines and spine fusion on head region for large (> 250 mm SL) specimens of *Scaphirhynchus platorynchus* (n = 5), *S. platorynchus* × *Scaphirhynchus albus* (n = 12), and *S. albus* (n = 2)

	0	1	2	3	0	1	2	3
	Left pre-orbital spine				Right pre-orbital spine			
S. platorynchus	1	4			1	2	2	
S. platorynchus × *S. albus*		7	5			8	3	1
S. albus		2			1	1		
	Left parietal spine				Right parietal spine			
S. platorynchus	1	4				5		
S. platorynchus × *S. albus*		12			1	11		
S. albus		2				2		
	Left post-temporal spine				Right post-temporal spine			
S. platorynchus		5				4	1	
S. platorynchus × *S. albus*	4	8			5	6	1	
S. albus		2				2		
	Left tabular spine				Right tabular spine			
S. platorynchus		3	2			3	1	1
S. platorynchus × *S. albus*		3	4	5		4	3	5
S. albus		2				2		

For all head spines: 0, spine absent; 1, present but completely fused, forming a ridge; 2, present and partially fused into ridge; 3, present and exposed.

raised in a hatchery on commercial fish food. The lack of a natural diet and the homogeneity of the habitat in this setting could have affected the morphology of the specimens (e.g. Hegrenes, 2001). For example, snout spines were missing from all specimens in this study. This unusual spine morphology may be a direct result of the food used and/or the environment during the growth and development of these sturgeons.

Even with these potential design flaws, our study provided a controlled test of the current character indices that are used to identify *Scaphirhynchus* specimens and that ultimately form a large part of the basis for management decisions. Our results indicate that current character indices do not correctly identify small specimens or combined sizes of *S. albus*, *S. platorynchus*, and their hybrid. All indices worked fairly well in identifying large *S. platorynchus* from the other ATUs, but mean values given by several authors for this species were not congruent with data from hatchery-reared specimens. Several indices failed to separate *S. albus* and hybrids in a plot of character index values (Carlson and Pflieger, 1981; Keenlyne et al., 1994), and even those that separated these

Table 15
Proportional measurements of head spines for small (< 250 mm SL) and large (> 250 mm SL) specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

	S. platorynchus					*S. platorynchus* × *S. albus*					*S. albus*				
Character	n	Min.	Max.	x	SD	n	Min.	Max.	x	SD	n	Min.	Max.	x	SD
< 250 mm SL															
Pre-orbital spine															
Left	7	5.4	9.3	7.3	1.6	20	3.8	12.8	6.4	2.2	9	2.8	7.3	4.3	1.5
Right	7	5.0	8.7	6.6	1.7	20	3.5	11.5	6.3	2.2	11	2.8	9.4	4.6	1.8
Parietal spine															
Left	7	6.1	36.3	18.6	11.8	22	6.5	28.5	14.8	6.9	12	11.1	15.3	13.2	1.3
Right	7	9.6	29.9	18.5	9.4	22	7.2	30.6	13.3	6.5	12	10.4	20.3	14.1	2.7
Post-temporal spine															
Left	6	5.1	7.9	6.3	1.0	17	2.6	8.0	5.6	1.6	12	3.2	9.4	4.6	1.6
Right	5	3.3	8.6	5.1	2.1	20	2.4	10.3	5.4	2.0	12	3.1	10.6	5.0	2.0
Tabular spine															
Left	7	9.3	17.8	12.7	3.7	22	3.1	21.4	11.2	4.3	12	7.5	14.0	10.2	1.7
Right	7	8.3	13.9	11.6	2.1	22	5.3	20.9	12.1	4.1	12	10.5	15.7	12.4	1.5
> 250 mm SL															
Pre-orbital spine															
Left	4	1.6	5.3	2.9	1.7	12	2.1	7.5	3.5	1.5	2	2.1	2.1	2.1	0.0
Right	4	2.3	5.1	3.8	1.2	12	1.9	6.7	3.6	1.5	1	3.4	3.4	3.4	–
Parietal spine															
Left	4	4.1	6.4	5.5	1.0	12	3.4	8.2	5.8	1.6	2	5.7	7.7	6.7	1.4
Right	5	3.8	5.4	4.6	0.6	11	3.6	8.4	6.2	1.6	2	3.8	6.1	4.9	1.6
Post-temporal spine															
Left	5	3.7	6.8	4.9	1.2	8	2.8	5.3	3.7	0.9	2	1.7	2.4	2.0	0.5
Right	5	4.3	6.2	5.3	0.7	7	2.7	5.8	3.5	1.1	2	2.4	3.6	3.0	0.9
Tabular spine															
Left	5	6.5	12.9	9.1	2.5	12	2.9	11.8	7.7	2.8	2	5.2	5.7	5.4	0.3
Right	5	7.9	9.9	9.0	0.8	12	3.7	12.9	7.8	2.4	2	4.6	6.6	5.6	1.4

All measurements expressed as thousandths of standard length. Disparity in sample sizes due to some specimens lacking spines.

Table 16
Statistically significant pairwise comparisons of mensural spine characters for small (< 250 mm SL) and large (> 250 mm SL) specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus* using SL as denominator (Kruskal–Wallis) or covariate (ANCOVA)

S. platorynchus/*S. albus*	*S. platorynchus*/hybrids	*S. albus*/hybrids
< 250 mm SL		
Kruskal–Wallis/ANCOVA		Kruskal–Wallis/ANCOVA
Pre-orbital spine right		Pre-orbital spine right
Kruskal–Wallis		Kruskal–Wallis
Pre-orbital spine left		Pre-orbital spine left
Post-temporal spine left		
> 250 mm SL		
ANCOVA	ANCOVA	ANCOVA
Post-temporal spine left	Parietal spine right	Parietal spine right
Post-temporal spine right	Post-temporal spine right	
Tabular spine right		

Characters are grouped as significant in both analyses or only significant in a single analysis.

Table 17
Position of outer barbel relative to inner barbel for all specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *S. albus*, and *S. albus*

	Outer barbel relative to inner barbel		
	Anterior	Even	Posterior
S. platorynchus	7	6	
S. platorynchus × *S. albus*		10	24
S. albus			14

ATUs had several specimens with scores well outside the putative range (Sheehan et al., 1999; USFWS, 2000; Wills et al., 2002). These deficiencies, when applied to wild sturgeon populations, could seriously overestimate the prevalence of hybrids, or conversely, could under-represent the actual extent of hybridization.

Sheared PCA of morphometric variables between small and large specimens of *Scaphirhynchus* (Fig. 10) clearly demonstrated differences in shape between size classes within the same ATU and supported previously proposed allometric differences (Bailey and Cross, 1954; Mayden and Kuhajda, 1996). Our study also revealed several meristic characters that significantly varied with size, as well as several qualitative characters such as squamation, barbel fringe, mouth papillae, and gill raker and spine morphology. Clearly, separation of specimens into appropriate size classes is essential before character indices or any other analyses are used for identification of or differentiation between ATUs.

Table 18
Number of spines at the posterior-lateral end of head for all specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

	Spines at the posterior-lateral end of head			
	Left		Right	
	1	2	1	2
S. platorynchus	10	2	9	3
S. platorynchus × *S. albus*	26	8	22	12
S. albus	10	4	10	4

The character indices tested here were based on specimens from the Missouri and upper Mississippi rivers, and the hatchery-reared specimens were from this same area (upper Missouri River), yet these indices assigned numerous specimens to the wrong ATU. Extending the use of the indices to sturgeon populations in the lower Mississippi River basin urges extreme caution because of demonstrated infraspecific geographic variation in North American *Scaphirynchus*. For example, differences in meristic data between specimens of *S. platorynchus* from the upper Mississippi and Red rivers rivaled the differences between *S. platorynchus* and *S. suttkusi* (Mayden and Kuhajda, 1996), and *S. albus* in the lower Mississippi River are morphologically more similar to *S. platorynchus* than are *S. albus* from the Upper Missouri River based on morphometric data (Murphy et al., 2007). Further, genetic sequence data indicated samples of both species of *Scaphirhynchus* from the upper Missouri and Atchafalaya rivers had genetic distances that were nearly as large as the genetic distance between species at each locality (Campton et al., 2000). The same pattern was observed using microsatellite data from *S. albus* (Tranah et al., 2001). Even a separate character index developed for the lower Mississippi River basin will likely be an inferior identification tool relative to a PCA, but if developed, it should be validated with progeny of *S. albus*, *S. platorynchus*, and *S. albus* × *S. platorynchus* from brood stock captured in the area.

Superficially, hybrids most closely resemble *S. albus* because these ATUs share easily recognized characters such as barbel placement and belly squamation. Additionally, these two ATUs were difficult to distinguish with any of the four character indices examined in this study. But hybrids overlapped with both parental species for many meristic characters, and multivariate analyses of these data indicate that hybrids are intermediate with respect to their parental species (Fig. 9a–c). Sheared PCA of morphometric data, which represented the overall shape of the specimens, indicates that hybrids are intermediate to their parental species for large specimens, but that small specimens are actually more similar in shape to *S. platorynchus* (Fig. 11a,b).

An increased variability of hybrid specimens relative to specimens of *S. platorynchus* and *S. albus* was not found, and because the variability within each ATU was similar, this study was able to uncover numerous characters that were significantly different among these entities. It has been suggested that hatchery-reared hybrids may exhibit less variation than their 'wild' counterparts due to the limited number of parents used in a hatchery setting (Leary et al., 1983), and therefore, these findings may not be applicable to wild *Scaphirhynchus* hybrids.

Many traditional as well as several new characters were found to differ between the ATUs. This included six meristic and 12 morphometric characters that were significant among ATUs in univariate analyses, loaded heavily on axes in multivariate analyses, and are likely practical for collecting data from in the field (Table 12), although ease of data collection remains to be tested. Half of these characters have not been used in character indices, and many others that have been used (e.g. outer barbel length and head length) were found to be of minimal use in differentiating between the three ATUs. PCA analyses of these data provided complete separation between ATUs in both small- and large-size classes, and because no *a priori* identification of a specimen is required, positive field identifications are not as critical. Head armature has not been used to differentiate between ATUs possibly due to the potential for spines to wear down over time. But head spines were typically larger in *S. platorynchus* relative to *S. albus* for both small as well as large-size classes (Tables 15 and 16), and should be considered as useful characters. Numerous qualitative characters are also extremely useful in differentiating among ATUs, especially for large-class specimens (Table 19). Most qualitative characters are easy to observe in the field and should be considered part of the data-collection protocol.

The ability to correctly identify live specimens of *S. albus* is essential for fisheries biologists studying this sturgeon or collecting specimens for brood stock. Reliable identification is critical when individuals are captured, tagged, and then released for studies on population estimates, habitat preference, and movement. Additionally, results of genetic studies using tissues from field-identified specimens that are released back into the wild are rendered essentially useless if the identity is questionable or inaccurate, an important problem existing in all current molecular analyses of *Scaphirhynchus*. This problem may explain in part the continuing difficulties in genetic

Table 19
Qualitative characters useful in differentiating between large-class (>250 mm SL) specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* × *Scaphirhynchus albus*, and *S. albus*

Character	*S. platorynchus*	*S. platorynchus* × *S. albus*	*S. albus*
Base of outer barbel relative to inner barbel	Anterior or even	Even or posterior	Posterior
Belly squamation	Mostly scaled	Mostly naked	Mostly naked
Dorsal–lateral squamation	Small embedded scales & light spicules may be present	Small embedded scales & light spicules may be present	Naked
Papillae on leading edge of barbel	Complex, branching	Intermediate	Simple, unbranching
Papillae on lip lobes	Long, thick, branching	Long, thick, branching	Simple & unbranching to almost absent
Gill rakers	Fan-like, malleable	Intermediate	Peg-like, stiff

analyses seeking to identify unique genetic markers for these species and efforts to understand population and phylogenetic relationships. Recording appropriate data from these released specimens (Tables 12 and 19) and making it available for scientific scrutiny is essential in order for researchers to have a scientific or legal basis for genetic or any other studies. This includes traditional recording of counts and measurements, but also assessment of appropriate qualitative characters and some form of vouchering, such as a visual record via photographs or videotape. Use of the methods and techniques presented herein on live sturgeon should permit a stronger confidence in the accuracy of specimen identification and the differentiation of *S. platorynchus*, *S. albus*, and their purported hybrids.

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