Final Report

Morphological Comparisons of Hatchery-Reared Specimens of *Scaphirhynchus albus*, *S. platorynchus*, and *S. albus* x *S. platorynchus* Hybrids

Grant Agreement No. 1448-60181-98-G616

Submitted to:

Steve Krentz
U.S. Fish and Wildlife Service
3425 Miriam Avenue
Bismarck, North Dakota 58501

Submitted by:

Bernard R. Kuhajda
Richard L. Mayden
Department of Biological Sciences
Box 870345
University of Alabama
Tuscaloosa, Alabama 35487-0345

20 September 2001

Abstract

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, *S. platorynchus*, causing hybridization between these two species. Several character indices have been developed to assist fisheries biologists in identifying specimens of *S. albus* from *S. platorynchus* and hybrids of these two species. Character 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 produce inaccurate identifications, then all previous work on status surveys, habitat use or migration studies, captive propagation efforts, or the harvesting of tissues for genetic studies are questionable. To test these indices, we examined progeny of "known" pallid, shovelnose, and hybrid sturgeon that U.S. Fish and Wildlife Service propagated, raised, and preserved at hatcheries. These 60 specimens (78-600 mm SL) were propagated with breeding stock from the upper Missouri River Drainage, where hybridization between these two species presumably does not occur. Results indicate that current indices do not correctly identify small (< 250 mm SL) or combined sizes of *S. albus*, *S. platorynchus*, and hybrid sturgeon. Indices work fairly well in identifying large (> 250 mm SL) *S. platorynchus*, but differentiating between large *S. albus* and hybrids was not realized. An alternative approach to character indices is principal components analysis (PCA). No a prior knowledge of the identity of the specimen is required with this multivariate technique, which avoids potential circular reasoning present in indices. We employed a standard PCA on a correlation matrix of 13

meristic characters and a sheared PCA on a covariance matrix on 51 morphometric variables. These analyses provided complete or almost complete separation between these sturgeon species and their hybrids. Additionally, we demonstrated that first generation hybrids are intermediate with respect to their parental species. Multivariate analyses with a reduced character set of 6 meristic and 12 morphometric variables also lead to accurate and reliable specimen identification. Recording appropriate data from released specimens and making it available is essential for researchers to have any scientific or legal basis for genetic or any other studies. Additional data recording via photographs or videotape are also advisable.

Introduction

Scaphirhynchus albus, the pallid sturgeon, is an endangered species ranging from the upper Missouri River in Montana to the lower Mississippi River in Louisiana (Federal Register 55 [September 6, 1990]: 36641-36647). Extensive habitat modifications have contributed greatly to the demise of this species. Reproduction has been reduced or eliminated through destruction or alteration of spawning habitats. These same alterations have presumably interfered with the reproductive isolating mechanisms between *S. albus* and the sympatric *S. platorynchus*, the shovelnose sturgeon, causing hybridization between these two species (U.S. Fish and Wildlife Service 1993). Hybrids 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 have been reported from the lower Mississippi River (Warren et al. 1986, U.S. Fish and Wildlife Service 1993), indicating that hybridization may occur throughout the range of *S. albus*.

Accurate field identification of specimens of endangered *S. albus* from *S. platorynchus* and hybrids of these two species is crucial for status surveys, habitat use or migration studies, captive propagation efforts, or the harvesting of tissues for genetic studies. If the identification of "pure" specimens of either species is incorrect, this compromises efforts in all of these areas. To assist fisheries biologists in these endeavors, several other cumulative character indices have been developed (Keenlyne et al. 1994; Sheehan et al. 1999; U. S. Fish and Wildlife Service 2000). Every 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 assumption that a sample contains pure strains of both parental species and that the hybrids are morphologically intermediate relative to the parental species. Neither of these assumptions has been adequately demonstrated in any previous morphological studies of sturgeon. Not all fish hybrids exhibit intermediacy between parental species (Leary et al. 1983), and the assumption that *S. albus* x *S. platorynchus* hybrids should be intermediate has been questioned (Campton et al. 2000) and has no empirical basis. Additionally, genetically mediated morphological variation can be expressed differently in hybrids relative to either parental species. This has resulted in some hybrids having more morphological variability than either parental species (Wilde and Echelle 1997). Therefore, 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 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). PCA transforms data to a linear combination of the original characters and maximizes the variance of all characters along the first principal component. The second principal component is uncorrelated (orthogonal) to the first and maximizes the remaining variance, and so on. 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 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 has several flaws. First, meristic and morphometric data were combined into one correlation matrix for analysis. Humphries et al. (1981) and Bookstein et al. (1985) have shown meristic and morphometric data should be analyzed separately. A covariance matrix is appropriate for morphometric variables and a correlation matrix is appropriate for meristic data. Additionally, size differences between specimens were addressed within the PCA by dividing morphometric characters by standard length. But this method can create spurious correlations, which in turn can inflate the first eigenvalue and change the magnitude and direction of coefficients on the various principal components (Atchley et al. 1976). It has been suggested 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. A superior method is to "shear" the size factor from the actual shape component of the data matrix of a PCA (Humphries et al. 1981, Bookstein et al. 1985).

Allometry plays another important role in sturgeon morphometrics. Bailey and Cross (1954) found differential growth between small (less than 250 mm standard length (SL)) and large (greater than 250 mm SL) specimens of *Scaphirhynchus* for several measurements. Mayden and Kuhajda (1996) noted that more measurements were significantly different between small and large *S. platorynchus* than either size class was compared to *S. suttkusi*, the Alabama sturgeon. Historically, character indices have been 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 (Open River Field Station biologists, Missouri Department of Conservation, personal communication), however, necessitates the need for accurate identification of small individuals to assist in identifying spawning or nursery sites for *S. albus*.

With the numerous shortcomings of using traditional morphological characters and character indices currently used to identify specimens of *Scaphirhynchus*, serious questions arise as to the accuracy of previous identifications of *S. albus*, *S. platorynchus*, and purported hybrids of these two species. Moreover, genetic work on these sturgeon species commonly relies on tissues harvested from specimens identified with these character indices, and specimens are typically released into the wild without any vouchering protocol. This methodology ultimately compromises any scientific analysis of genetic data and any investigations on species boundaries of these sturgeons.

Recent attempts to verify the accuracy of character indices by using molecular techniques have revealed some differences at a regional scale for *S. albus* and *S. platorynchus* (Campton et al. 2000). However, the search for unique genetic markers to distinguish between *S. albus* and *S. platorynchus* throughout their ranges or to identify hybrids of these two species has been unsuccessful. These techniques have included mtDNA haplotypes via DNA sequencing (Campton et al. 2000, Simons et al. 2001) and nuclear haplotypes using microsatellite loci (McQuown et al. 2000). The inability of these techniques to differentiate between *S. albus*, *S. platorynchus*, and there purported hybrids has been attributed to either slow rates of divergence between the two species at these markers, a very recent speciation event, or the prevalence of hybridization. However, as mentioned above, another major contributing variable to the inability to identify species-specific genetic markers is the likely misidentification of specimens using traditional morphological methods. 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. Given a more rigorous examination of morphological data and the quantification of morphological variation for species identification, the current uncertainty with molecular markers may be resolved.

In an attempt to address these hybrid issues, we examined progeny of *S. albus*, *S. platorynchus*, and *S. albus* x *S. platorynchus* hybrids, all propagated and raised at fish hatcheries. These data were used to examine the accuracy of the various character indices, both for small, large, and for all sizes of specimens. Univariate and multivariate (PCA) analyses were employed to investigate the usefulness of traditional as well as alternative characters in distinguishing between known specimens of *S. albus*, *S. platorynchus*, and there hybrids, and to determine if the these known hybrids were in fact intermediate with respect to the parental species.

Methods

The U.S. Fish and Wildlife Service (USFWS) propagated and raised specimens of *Scaphirhynchus albus*, *S. platorynchus*, and *S. albus* x *S. platorynchus* hybrids at Miles City State Fish Hatchery, Montana and Gavins Point National Fish Hatchery, South Dakota. All of the 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 then 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 included in this morphological 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, these two parental species of sturgeon and their hybrids will be referred to as analytical taxonomic units (ATUs).

We evaluated the accuracy of four character indices (Carlson and Pflieger 1981, Keenlyne et al. 1994; Sheehan et al. 1999; U. S. Fish and Wildlife Service 2000) in distinguishing between ATUs by analyzing data from up to four meristic and nine morphometric characters from these hatchery-reared specimens. We followed the methods of these indices with the exception of recording the length of the longest barbel rather than the mean length of both barbels, as was done by Sheehan et al. (1999). Additional data were obtained from hatchery-reared specimens for our more detailed morphological study. Methods for most counts and measurements follow those of Hubbs and Lagler (1958), Bailey and Cross (1954), Williams and Clemmer (1991), and Mayden and Kuhajda (1996). Some measurements (fifth dorsal plate and spine) are defined here for the first time. All meristic and morphometric data were taken from the left side of specimens except for medial structures and spines.

Meristics (13 characters): Dorsal plates anterior to dorsal fin include postoccipital plate (usually the first dorsal plate with a well formed spine and or keel) posteriad to second predorsal plate; the plate without a keel just anterior to dorsal fin was not counted. Dorsal plates posterior to dorsal fin include first plate lateral to posterior edge of dorsal-fin base posteriad to single dorsal plate at base of caudal fin. Lateral plates include plate just behind shoulder girdle (even if it was without a ridge) posteriad to last keeled plate. Lateral plates anterior to dorsal fin include plate which had any part intersected by a vertical line through dorsal-fin origin anteriad to first lateral plate. Ventral-lateral plates include plate just anterior to pelvic fin anteriad to first keeled plate. Plates between anus and anal fin include first plate lateral to posterior edge of anus posteriad to single preanal plate. Ventral plates posterior to anal fin include first plate lateral to posterior edge of anal-fin base posteriad to single ventral plate at base of caudal fin. Dorsal and anal-fin rays include all anterior rudiments behind predorsal or preanal plates; the last ray is split at base. Pectoral-fin rays include the anterior spine and all posterior rudiments; pelvic-fin rays include all anterior rudiments. 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. Gill rakers include all structures with ends noticeably free from surrounding tissues on the first arch. Gill-raker tips include all structures with ends noticeably free from surrounding tissues of the gill raker.

Morphometrics (52 characters): Standardizing measurements include standard length (tip of snout to posterior edge of last keeled lateral plate) and fork length (snout to caudal-fin fork). Morphometric characters include snout to dorsal-fin origin (posterior edge of predorsal plate), snout to pelvic-fin insertion, snout to pectoral-fin insertion, head length (snout to bony posterior edge of operculum), snout to most anterior edge of operculum, snout to tip of spine at posterior-lateral head edge, snout to anterior edge of orbit, snout to anterior edge of anterior nostril, snout to occiput, pectoral-fin to pelvic-fin insertion, pectoral-fin length, pectoral-fin insertion to occiput, body depth at pectoral-fin insertion (includes ridge or spine of dorsal plate), head depth at anterior edge of parietal ridge, head depth at anterior edge of anterior nostril, pelvic-fin length, pelvic-fin insertion to anal-fin origin (posterior edge of preanal plate), pelvic-fin insertion to dorsal-fin origin, dorsal-fin length, dorsal-fin base, anal-fin to dorsal-fin origin, anal-fin origin to posterior edge of 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, anal-fin base, caudal peduncle depth (least depth), caudal peduncle width (just ventral to lateral ridge or spine at anterior edge of precaudal plate), tenth lateral plate height (measured at plate angle), fifth dorsal plate and spine length (anterior edge of plate to tip of spine), fifth dorsal plate length (anterior edge of plate to posterior base of spine), fifth dorsal plate and spine height (ventral edge of plate to highest point of plate or spine), fifth dorsal spine height (dorsal edge of tip of fifth dorsal spine ventrally to sixth dorsal plate directly below), interorbital width, orbit length, posterior nostril width, anterior nostril width, pectoral girdle width (just anterior to pectoral-fin insertion), anterior edge of mouth (midline of anterior cartilage edge of labial depression) to pectoral-fin insertion, anterior edge of mouth to snout, anterior edge of mouth to anterior base of inner barbel, anterior edge of mouth to anterior base of outer barbel, anterior edge of mouth to left head edge even with base of outer barbel, snout to anterior base of inner barbel, snout to anterior base of outer barbel, snout to left head edge lateral to anterior edge of mouth, longest outer barbel length (anterior edge of base to tip), longest inner barbel length, head width at outer barbel bases, head width at anterior edge of mouth, head width at tip of spine at posterior-lateral head edge, head width at widest point, and mouth width (widest measurement on outer edge of lips).

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 preorbital, parietal, posttemporal, 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. Spines at posterior-lateral head edge were counted but no measurements were taken. Other characters recorded included placement of outer barbel relative to inner barbel, dorsal-lateral, ventral-lateral, and belly squamation, spine size on most posterior ventral-lateral plate with respect to other ventral-lateral plate spines, anterior extent of complete armor on caudal peduncle, presence of a belly ridge, gill raker shape and rigidity, development of lip papillae and barbel fringe, fin pigment pattern (uniform or light-edged), and overall body color.

For all analyses SAS (Cary, NC, 1989-1996, 1999) and DataDesk (Ithaca, NY, 1997) were employed; statistical significance occurred for $P < 0.05$. All data were examined for normal distributions and homogeneity of variance within each ATU. All data were determined to be nonparametric (see details below), therefore the Kruskal-Wallis test was employed and *post-hoc* tests used pairwise comparisons of the Wilcoxon rank sum test. The probability level for all pairwise comparisons was adjusted using the Bonferroni technique. Because *S. albus* has a different head shape relative to *S. platorynchus* (Bailey and Cross 1954), neither head length or 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 with SL. Sexual dimorphism within ATUs was not explored because specimens were juveniles or small non-reproductive adults.

We tested the independence of meristic characters with respect to size by examining the correlation between SL and each meristic character. Those characters found to vary significantly with size were divided into size classes and analyzed separately. We also examined the assumed dependence of morphometric data with size. Fifth dorsal spine height did not significantly co-vary with SL, either for small or large specimens across or within ATUs. This variable was dropped from all subsequent analyses. Fifth dorsal plate length did not demonstrate a significant correlation with SL within small *S. albus*, and 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.

Morphometric data must be adjusted to compensate for size differences between specimens. The use of ratios in univariate analyses is common, but this method has been shown to produce spurious results (Jackson and Somers 1991) and its critics recommend using SL as the covariate in an analysis of covariance (ANCOVA). But others do not consider ratios a problem (Prairie and Bird 1989) and some point to shortcoming of ANCOVA (Sokal and Rohlf 1981). To address these issues, we used both methods for our univariate analyses. Ratios were arcsine transformed and raw measurements were log10 transformed in an attempt to improve normality and/or homogeneity of variance over untransformed data. Arcsine-transformed ratios did improve the homogeneity of variance and were used in Kruskal-Wallis rank sum tests, but no improvement was noted with the log10 transformation. The log10 transformation also failed to improve the linearity of the data for the ANCOVA; therefore untransformed measurements were used in these analyses. An ANCOVA was not run for the following morphometric characters because the homogeneity of slopes assumption was not satisfied: small specimens include 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 base, and outer barbel length; large specimens include anterior mouth to pectoral-fin insertion. The coefficient of variation (CV) was used to compare the variability of morphometric ratios between ATUs.

All multivariate analyses of morphometric data employed sheared PCA on 51 untransformed characters (D. L. Swofford, SAS Program for computing sheared PCA, unpubl., 1984, privately distributed). This method removes size variation among specimens along the first principal component, therefore only shape differences are expressed along the second and third components. To determine if our hatchery-raised specimens exhibited similar allometric growth patterns as described by Bailey and Cross (1953) and Mayden and Kuhajda (1996), we employed sheared PCA on a covariance matrix containing both small (less than 250 mm SL) and large (greater than 250 mm SL) specimens across all ATUs. We found complete or substantial separation between groups of different sized specimens within each ATU (see Results), therefore all subsequent morphometric analyses were computed separately on small and large size classes as defined above. To summarize meristic variation within and between ATUs, we employed PCA using a correlation matrix on all 13 characters. Because there were only four characters that were size dependant, it was not evident whether or not to separate this analysis into size classes, therefore the analysis was computed both with two size classes as defined above and with combined sizes.

Results

Character indices – We examined the effect of sample size and geographic scale on character indices by using data from Keenlyne et al. (1994). In their study specimens of *Scaphirhynchus albus* and *S. platorynchus* were examined from three separate reservoirs on the upper Missouri River in Montana and North and South Dakota. They 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 with 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. Separate analyses of each isolated population revealed that specimens from the headwaters of the two upper reservoirs (Fort Peck and Garrison) had a distinct separation between species (Figures 1 and 2). In the population from the lower reservoir (Lake Sharpe), three specimens field identified as *S. albus* had index values below 300 (Figure 3) and were considered specimens of questionable purity. But how would the index change if this had been a study on a larger geographic scale and all three populations in the upper half of the Missouri River (293 specimens) were considered one population? Those same three purported hybrid specimens (as well as a fourth) would be classified as *S. platorynchus* and no potential hybrids would be evident, or several specimens of *S. platorynchus* with the same scores as the purported hybrids would also be suspect (Figure 4). This illustrates the inherent susceptibility of character indices to the influences of sample size or to the scale of the question being asked.

Our hatchery specimens were used to test the various character indices that fisheries biologists commonly employ to distinguish these three ATUs. When data from all 60 hatchery-reared specimens were evaluated together (regardless of size) using the formula comprised of morphometric variables from Keenlyne et al. (1994), hybrids overlapped minimally with *S. platorynchus* but were indistinguishable from *S. albus* (Figure 5). In fact the specimen with the highest *S. albus*-like score was a hybrid. The same pattern appeared when both size classes were evaluated independently; hybrids overlapped only slightly (small size class) or not at all (large size class) with *S. platorynchus* but overlapped completely with *S. albus* (Figures 6 and 7).

The U.S. Fish and Wildlife Service (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 provide these values based on 262 specimens from the upper Missouri River. If the user's sample falls outside of the range of these minimum or maximum values, then the new values should be used. Our sample produced two minimum values below those given, and we adjusted the formulae accordingly. Significant overlap was prevalent between hybrids and *S. albus* for both combined and small size classes, but several specimens of *S. albus* scored higher than any hybrids (Figures 8 and 9). For the large size class, *S. albus* was separate from hybrids (Figure 10), but the mean score of 414 for *S. albus* was far below the 514 reported by U.S. Fish and Wildlife Service (2000). Hybrids plotted completely separate from *S. platorynchus* in all size comparisons (Figures 8, 9, and 10), but the mean scores of 287-294 for *S. platorynchus* were much greater than the reported value of 230. The overall improvement in separating *S. albus* from hybrids with the character index from U.S. Fish and Wildlife (2000) can be attributed to the minimum and maximum values that were provided. When we used minimum and maximum values from our sample in all calculations rather than those provided, the partial or complete separation between *S. albus* and hybrids was gone for all size comparisons (Figures 11, 12, and 13). This method did, however, create mean scores of 440-467 for *S. albus*, which were closer to the reported mean of 514. For *S. platorynchus*, the means for the combined and small size classes were 229 and 240 respectively, which conformed to the reported mean of 230.

While the previous two character indices rely solely on measurements, the remaining two indices have meristic components. 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 operculum), 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. Using data from our hatchery-raised specimens with their index, *S. platorynchus* separated almost completely from hybrids, only one specimen of *S. platorynchus* plotted within hybrids for the combined and small size classes and there was complete separation for the large size class (Figures 14, 15, and 16). Specimens of *S. albus* all scored above the middle of the index, but about half of the hybrids had similar scores. For the large size class, one hybrid specimen actually scored higher than any *S. albus* (Figure 16). Because it is difficult to count fin rays on live specimens, we examined the usefulness of the index by Carlson and Pflieger (1981) using only their nine 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 (Figures 17 and 18). Additionally, complete overlap occurred between hybrids and *S. albus* for all comparisons only using morphometric data, with hybrids having the highest scores, and many *S. albus* had scores below the middle of the scale (Figures 17, 18, and 19).

The character index developed by Sheehan et al. (1999) is based on data derived from the 30 specimens identified by and presented in Carlson and Pflieger (1981). Five ratios of five measurements (head length, mouth to inner barbel base, snout to outer barbel base, and inner and outer barbel lengths) 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. Sheehan et al. (1999) also produced an alternative equation using only data from the five measurements because of the difficulty in obtaining fin-ray counts in the field. Using their morphometric index with data from our combined and small size class specimens, minimal overlap occurred between hybrids and *S. platorynchus*, but hybrids and *S. albus* overlapped extensively (Figures 21 and 22). There was complete separation between ATUs for the large size class, but several hybrids scored below the given mean of -0.69 for *S. albus* (Sheehan et al. 1999) (Figure 23).

Their morphometric and meristic index produced similar separation of hybrids from *S. platorynchus* for our combined and small size classes; overlap of hybrids with *S. albus* was not as extensive but still prevalent (Figures 23 and 24). Similar results were also obtained for the large size class; complete separation between all three ATUs, but several hybrids scored at or below the mean of -0.86 given for *S. albus* (Figure 25).

A total of nine morphometric and four meristic characters are used by the above mentioned character indices to differentiate between these three ATUs. We analyzed data from 51 morphometric and 13 meristic characters in an attempt to evaluate the existing diagnostic characters and to possibly uncover other useful characters in differentiating between these ATUs.

<u>Meristic analyses</u> –Most researchers assume that the size of post-larval specimens of fishes does not influence meristic data. Because accurate identification of small specimens of *Scaphirhynchus* is vital for differentiating the breeding and nursery areas for each species, and because meristic characters are extremely useful for identification of small specimens, we tested this assumption by examining the correlation between SL and meristic characters. Nine characters had a significant correlation with SL within or across all ATUs (Table 1). Four of these characters had a significant correlation within only one ATU, and pelvic-fin rays had no correlation with $P < 0.01$, therefore all sizes were analyzed together for these characters. The remaining four characters were divided by size class for comparisons between ATUs. Number of lateral plates had a significant negative correlation with size for all ATUs combined (Figure 26). Because there was no correlation with SL for number of lateral plates anterior to the dorsal fin, the higher number of plates for smaller specimens can be attributed to keeled lateral plates

extending further onto the caudal peduncle. One might assume that as all *Scaphirhynchus* increase in size, the posterior-most keels wear down and the number of lateral plates decreases. However, hybrids were the only ATU to have a significant correlation (Table 1, Figure 27), so this result was not necessarily artifact. This was perhaps not the case for pectoral-fin rays; the decrease in fin ray number with larger specimens occurred in all ATUs (Figure 28), and the small rays on larger specimens are more difficult to count because of the thicker tissue on the fins. The number of gill rakers and gill-raker tips increased significantly for *S. platorynchus* and hybrids, but specimens of *S. albus* did not show this relationship (Figures 29 and 30).

Several significant meristic differences were found between ATUs within each size class. All three ATUs differed significantly for pectoral-fin rays for the small size class, and no overlap existed between *S. platorynchus* and *S. albus* (Table 2). 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 two ATUs. All small *Scaphirhynchus* had similar numbers of gill-raker tips (Table 2). Because of the low sample size for large *S. albus*, there were no significant differences found between this ATU and the other two for any of these four meristic characters, even though no overlap existed between *S. albus* and *S. platorynchus*. Significant differences between *S. platorynchus* and hybrids included pectoral-fin rays, gill rakers, and gill-raker tips (Table 2).

The analyses of meristic data which compared ATUs regardless of size class disclosed four characters which differed significantly between all three ATUs; number of dorsal plates posterior to dorsal fin and 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 3). *Scaphirhynchus albus* was significantly different from the other ATUs for number of ventral plates posterior to anal fin and from *S. platorynchus* for ventral-lateral plate counts; *S. platorynchus* differed significantly from hybrids for number of lateral plates anterior to dorsal fin (Table 3).

PCA of all 13 meristic characters provided complete separation between ATUs along PC1 for both small and large size classes, and almost complete separation between ATUs along this same axis for all sizes combined (Figures 31, 32, and 33). PC1 accounted for 43 to 44 percent of the variation in all analyses. Factors that had heavy positive loadings along PC1 for all combined sizes were those same variables that were significant between all three ATUs (all fin-ray counts and number of dorsal plates posterior to dorsal fin) as well as number of lateral plates. Factors that had heavy negative loadings included number of gill rakers and gill-raker tips (Table 4).

Known *Scaphirhynchus* hybrids had intermediate meristic characters relative to their parental species. Hybrids had means between *S. platorynchus* and *S. albus* in 13 of 17 univariate comparisons of meristics,. Likewise, 9 of 17 hybrids had modes for meristic variables that were intermediate between the parental species, while modes of four other variables were shared between all three ATUs (Tables 2 and 3). Although meristic values for hybrid specimens overlapped with the range of both parental species in all but one comparison (gill rakers of large specimens versus *S. albus*), there were several significant differences noted above. PCA of both size classes and combined sizes showed hybrids as intermediate between the two parental species (Figures 31, 32, and 33).

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 2 and 3). Likewise in the PCA plots, the size of the polygons for hybrids was only slightly larger than that of *S. platorynchus*, even though the sample size for hybrids was larger (Figures 31, 32, and 33). Because of the similar variability of these meristic characters across all ATUs, hybrids are readily distinguishable from their parental species.

<u>Morphometric analyses</u> – To determine if hatchery-raised specimens exhibited similar allometric growth patterns as described by Bailey and Cross (1953) and Mayden and Kuhajda (1996), we ran sheared PCA on both small and large specimens across all ATUs. We found complete separation between size classes of *S. albus*, and substantial separation between different sizes of *S. platorynchus* and hybrids (Figure 34). These results demonstrate that allometry occurs between different sizes of ATUs, therefore all subsequent morphometric analyses were run separately on small and large size classes.

Proportional measurements for both small and large size classes of each ATU are presented in Table 5 as thousandth of SL. Pairwise comparisons between ATUs for these measurements revealed numerous significant differences within each size class (Tables 6 and 7). Even though seven measurements were not used with ANCOVA (these did not meet the homogeneity of variance assumption), this analysis produced more significant results than did Kruskal-Wallis for all pairwise comparisons except *S. albus* / hybrids within the small size class. Both analyses 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 the three ATUs within the small size class

(Table 6). Of the seven measurements not examined with an ANCOVA, snout to base of outer barbel was significantly different across pairwise comparisons using the Kruskal-Wallis analysis (Table 6). 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 5). Because of the low number of *S. albus* for the large size class, no significance was found when this species was compared to the other two ATUs using the ranked sum test (Kruskal-Wallis) (Table 7). Within the ANCOVA analysis, variables that were significant between all three multiple comparisons of ATUs included anterior edge of mouth to base of outer barbel, anterior edge of mouth to head edge outer barbel, and inner barbel length (Table 7). The low sample size of large *S. albus* also lead to numerous ranges of proportional measurements for this species not overlapping 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 5).

Several variables were significantly different within a pairwise comparison of ATUs for both small and large specimens. For the comparison of *S. platorynchus* with *S. albus* (ANCOVA only) dorsal-fin base, anal-fin to dorsal-fin origin, anterior edge of mouth to base of inner and to base of outer barbel, anterior edge of mouth to head edge at outer barbel, inner barbel length, head width at anterior edge of mouth, and mouth width were significant (Tables 6 and 7). Comparisons of *S. albus* with hybrids (ANCOVA only) revealed that snout to pectoral-fin insertion, head length, snout to tip of spine at head end, anterior edge of mouth to base of inner and to base of outer barbel, anterior edge of mouth to head edge at outer barbel, snout to head edge at anterior edge of mouth, and inner barbel length were significant across both size classes (Tables 6 and 7). For the comparison of *S. platorynchus* and hybrids, both ratio and covariate analyses found anterior edge of mouth to base of outer barbel and inner barbel length significant. Additionally, within the ranked sum test, snout to base of inner and to base of outer barbel shared significance between both size classes. The ANCOVA analysis found snout to pectoral-fin insertion, head length, snout to anterior edge operculum and to anterior edge of orbit, snout to anterior edge of anterior nostril and to occiput, anal-fin to dorsal-fin origin, anterior mouth to head edge outer barbel, snout to head edge at anterior mouth, head width at outer barbel, and mouth width as significant variables for both small and large specimens (Tables 6 and 7).

Sheared PCA of all 51 morphometric characters of small size class specimens showed complete separation of *S. albus* from the other two ATUs along PC2 (Figure 35). Orbit length, anterior edge of mouth to base of inner and to base of outer barbel, and inner barbel length were the positive factors which loaded heavily along PC2, while those negative factors loading heavily included head depth anterior to parietal ridge, dorsal-fin base, and anal-fin to dorsal-fin origin (Table 8). There was only moderate separation between *S. platorynchus* and hybrids along both PC2 and PC3 (Figure 35). Characters with heavy positive loadings along PC3 include anterior nostril width and snout to base of outer barbel, while heavy negative loadings included tenth lateral plate height, fifth dorsal plate and dorsal plate and spine length (Table 8). Complete separation between all three ATUs was realized along the sheared PC2 axis for large specimens (Figure 36). Factors with heavy positive loadings included dorsal-fin base, anterior nostril width, and snout to base of outer barbel, while negative factors loading heavily included fifth dorsal plate and 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 9).

Known hybrids within the small size class had 17 proportional measurement means with values intermediate compared to their parental species, but a total of 21 means had closer affinities with *S. platorynchus* compared to only 10 with *S. albus* (Table 5). This was evident in the sheared PCA for small specimens, where hybrids and *S. platorynchus* overlapped (Figure 35). 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 5), the sheared PCA shows hybrids were intermediate in shape relative to the parental species (Figure 36).

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 5). In 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 5). But even with this higher variability, there was complete separation of hybrids from their parental species within the sheared PCA (Figure 36).

<u>Combined axes from meristic and morphometric PCA</u>: – As noted above, complete separation was realized in both size classes along PC1 within a PCA of meristic data (Figures 31 and 32). Separation between ATUs using morphometric data was evident along sheared PC2 (Figures 35 and 36). Combining these two axes into one plot

maximized the differentiation between *S. platorynchus*, *S. albus*, and hybrids (Figures 37 and 38). Only slight overlap occurred within the small size class between *S. platorynchus* and hybrids; elsewhere, complete separation between ATUs was realized (Figures 37 and 38).

The above plots represent 13 meristic and 51 morphometric variables that were recorded from preserved specimens. It is not feasible to record all of these data from live specimens of *Scaphirhynchus*, and the endangered status of *S. albus* dictates that minimal time be spent with data collection. We therefore employed separate PCAs for both meristic and morphometric data on reduced data sets of characters that were significantly different between ATUs or loaded heavily along the axes that separated ATUs. We excluded pectoral and pelvic-fin rays 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. Meristic data that were used in a PCA to produce the values for specimens along PC1 in the combined PCA plot therefore included only six counts: dorsal plates posterior to dorsal fin, lateral plates, lateral plates anterior to dorsal fin, ventral plates posterior to anal fin, and dorsal and anal-fin rays. Twelve significant measurements were used in a sheared PCA to produce values for specimens along sheared PC2 in the combined PCA plot: head depth anterior to parietal ridge, dorsal-fin base, anal fin to dorsal fin origin, fifth dorsal plate and spine length, orbit length, anterior edge of mouth to base of inner and to base of outer barbel, anterior edge of mouth to head edge at outer barbel, snout to base of outer barbel, inner barbel length, head width at anterior edge of mouth, and mouth width.

Results of a combined plot of PCA axes from meristic and morphometric analyses on this reduced data set gave complete separation between the three ATUs for both small and large specimens (Figures 39 and 40). Excluding the above mentioned significant counts did reduce the separation of ATUs along PC1 for both size classes. A slight decrease in separation was also noted along the sheared PC2 axis for the large size class (Figure 40), but using only the significant morphometric characters actually increased the separation within the small size class between *S. platorynchus* and hybrids (Figure 39).

Spine characters – All 60 specimens of hatchery-reared *Scaphirhynchus* lacked spines on their snout. This was unexpected since snout spines are typically present in wild caught *S. platorynchus* and *S. albus* (Bailey and Cross 1954, Mayden and Kuhajda 1996). All other head spines were present in most specimens. One hybrid specimen had the left parietal spine bifurcate, another had the left tabular spine bifurcate and the right tabular area represented by three spines. Eight additional specimens representing all three ATUs had the right tabular spine present as two spines. All of these specimens with multiple spines were less than 200 mm SL, suggesting that these spines may fuse as specimens get larger.

We tested the relationship of SL with degree of spine fusion and found a significant correlation for all eight characters when all three ATUs were examined together. As specimens increased in size, the spine tended to become fused into a ridge or to be absent. Examination of this correlation within each ATU revealed that preorbital and parietal spine fusion varied significantly with SL for *S. platorynchus* and hybrids, but not *S. albus* (Figures 41 and 42), whereas *S. albus* varied significantly for posttemporal spine fusion but the other two ATUs did not (Figure 43). Tabular spine fusion varied significantly within all ATUs (Figure 44). 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 very similar (Table 10). Most specimens of *S. platorynchus* and hybrids had preorbital spines exposed or only partially fused, while most specimens of *S. albus* had these spines completely fused or missing. Conversely, most specimens of *S. albus* had prominent posttemporal spines, while numerous *S. platorynchus* had these spines absent or fused (Table 10). For the large size class, all specimens had tabular spines present and only one or two specimens were missing preorbital and parietal spines (Table 11). Several hybrid specimens lacked posttemporal spines, and these spines were poorly developed in all ATUs (Table 11).

Proportional measurements of head spines for both small and large size classes are presented in Table 12 as thousandth of SL. The disparity in sample sizes is due to some specimens not having spines to measure. Pairwise comparisons between ATUs for these spine measurements revealed several significant differences within each size class (Table 13). Within the small size class, *S. albus* had significantly smaller right preorbital spines than either *S. platorynchus* or hybrids for both univariate analyses and possessed a smaller left preorbital spine than either ATU for the Kruskal-Wallis test. The left posttemporal spine of *S. albus* was also significantly smaller than that *S. platorynchus* (Tables 12 and 13). Only the ANCOVA showed any significant differences in spine size within the large size class. Both posttemporal spines and the right tabular spine were significantly smaller in *S. albus* relative to *S. platorynchus*, and the right posttemporal spine of hybrids were also smaller than that in *S. platorynchus*. Hybrids had significantly larger right parietal spines relative to the other ATUs (Tables 12 and 13).

As with morphometric characters of the head and body, spine size for hybrids was typically intermediate relative to the parental species in most cases. For both small and large size classes, six of eight spine characters of hybrids had means that were intermediate (Table 12).

<u>Additional characters</u> – Several other character sets were examined during this study. Bailey and Cross (1954) noted that 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. This character was diagnostic for these two species in this study, and hybrids had outer barbels either even or posterior to inner barbels (Table 14). All hybrids greater than 300 mm SL had outer barbels posterior to inner barbels.

Another diagnostic character examined by Bailey and Cross (1957) was belly squamation of adults. In *S. platorynchus* the belly is mostly scaled and in *S. albus* it is mostly naked. Four of five large size class specimens of *S. platorynchus* followed this pattern, and all large *S. albus* and hybrids had naked bellies. Bailey and Cross (1954) found that this character was not useful for smaller *S. platorynchus*, and five of our seven small specimens lacked belly squamation, as did all small *S. albus* and hybrids.

We also noted the extent of squamation on the dorsal-lateral and ventral-lateral areas between rows of plates. 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 greater than 300 mm SL had small, embedded scales as well as light spicules present. Other specimens were similar to *S. albus*. No specimens had any scales present in the ventral-lateral area. All specimens of *S. platorynchus* had spicules across this area, but most specimens of *S. albus* and hybrids had reduced spicules or none at all.

Bailey and Cross (1954) noted that the barbel fringe on *S. platorynchus* was better developed relative to *S. albus*, a pattern of variation also observed in this study. 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 greater than 100 mm SL had mostly simple unbranched papillae on the leading edge, but had branching on the other papillae; specimens less than 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 (less than 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 specimens, 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 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 less than

100 mm SL had much smaller, fewer, and simpler papillae. These simple papillae were the only type present in *S. albus*, and 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 the complex and large papillae and no specimens had papillae reduced to knobs as in *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 (see above) and are more fan-like relative to S. *albus*. We observed this same pattern in our specimens and also noted that the gill rakers in *S. albus* were stiff and remain erect in preserved specimens. Gill rakers 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 (less than 165 mm SL) had reduced armature, while seven (greater than 175 mm SL) had more extensive caudal peduncle armor.

Numerous specimens in all three ATUs had two rather than one spine present at the posterior-lateral end of the head (Table 15). All of these specimens, except one *S. platorynchus*, were in the small size class (less than 250 mm SL). 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; two hybrids had this spine slightly smaller. Nearly half (nine) of the specimens in the large size class had this spine worn off, specimens with spines present 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 as well as 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 two size classes or between the three ATUs.

Discussion

This study is the first to use a data set obtained from hatchery-reared specimens of *Scaphirhynchus* representing “known” *S. platorynchus*, *S. albus*, and hybrids from these two parental species. Although the use of specimens bred in a controlled environment has numerous advantages over using wild-caught specimens in addressing the various issues presented here, especially for hybrids, there were several shortcomings using hatchery-reared specimens. Our sample size was small for some ATUs, especially for the large size class. Additionally, the brood stock all came from the extreme upper Missouri River, so the geographic coverage was extremely limited. Since there is no method to establish “pure” *Scaphirhynchus albus* and *S. platorynchus*, we could only assume that the stocks were pure due to the lack of reported hybrids from this part of the drainage. Only a one-way cross (two male *S. albus* x 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 raised in a hatchery on commercial fish food. The lack of a natural diet and the homogeneity of the habitat in this setting could affect the morphology of the specimens. It was noted that snout spines were missing from all specimens in this study. This unusual spine morphology may have been a direct result of the food used and/or the environment present during the growth and development of these sturgeons.

Even with these potential design flaws, this study is an excellent vehicle to test the current character indices that are used to identify specimens in status, habitat use, or migrations studies, captive propagation efforts, and harvesting of tissues for genetic studies. Our results indicate that current character indices do not correctly identify small specimens or combined sizes of *S. albus*, *S. platorynchus*, and hybrid sturgeon. All indices work fairly well in identifying large *S. platorynchus* from the other two ATUs, but mean values given by several authors for this species were not realized with our data. Several indices fail to separate *S. albus* and hybrids in a plot of character index values (Keenlyne et al. 1994, Carlson and Pflieger 1981), and even those that can separate these two ATUs have several specimens with scores well outside their given range (Sheehan et al. 1999, U.S. Fish and Wildlife Service 2000). The deficiencies of these character indices could give the impression that hybrids are much more prevalent than they actually are, or conversely, could under represent a notable hybrid problem.

The presence of allometry between size classes within *Scaphirhynchus* has been examined by Bailey and Cross (1954) and Mayden and Kuhajda (1996). Our sheared PCA of morphometric variables between small and large specimens (Figure 34) clearly demonstrates differences in shape between size classes within the same ATU. We also found several meristic characters that significantly varied with size, as well as dorsal-lateral, ventral-lateral, and belly squamation, development of a belly ridge, barbel fringe, mouth papillae, and gill raker and spine morphology. It is essential to separate specimens into appropriate size classes before character indices or any other analyses are used for identification of or differentiation between these ATUs.

Although Bailey and Cross (1954) did not address geographic variation within *Scaphirhynchus*, other studies have recently examined this issue. Mayden and Kuhajda (1996) demonstrated geographic variation in some meristic data between specimens of *S. platorynchus* from the upper Mississippi and Red rivers that rivaled the differences between this species and *S. suttkusi*. Campton et al. (2000) noted genetic differences between samples from the upper Missouri and Atchafalaya rivers for both species of *Scaphirhynchus* that were nearly as large as the genetic distance between species at each locality. All of the character indices we tested were based on specimens from the Missouri and upper Mississippi rivers, and our hatchery-reared specimens are from this same area (upper Missouri River), yet these indices assigned numerous specimens to the wrong ATU. Using these indices on specimens from the lower Mississippi River basin would have to be approached with extreme caution because of the demonstrated geographic variation within each species. A separate character index should be developed for the lower Mississippi River basin, and progeny of *S. albus*, *S. platorynchus*, and *S. albus* x *S. platorynchus* from brood stock captured in this area would be an excellent means to accurately test such an index.

Superficially, hybrids most closely resemble *S. albus* based on these two ATUs sharing such easily recognized characters 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 almost all meristic characters, and multivariate analyses of these data indicate that hybrids are intermediate with respect to their parental species (Figures 31, 32, and 33). 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, and small specimens actually have a more similar shape to *S. platorynchus* (Figures 35 and 36).

We did not find increased variability of hybrid specimens relative to specimens of *S. platorynchus* and *S. albus*. Leary et al. (1983) suggest that hatchery-reared hybrids may exhibit less variation than their "wild" counterparts due to the limited number of parents used in a hatchery setting, but specimens of the parental species we used for comparison were hatchery-reared in the same manner. The similarity in variability between these three ATUs was an important component of the data that allowed us to uncover numerous characters that were significantly different among these entities.

Many traditional as well as several new characters were found to differ significantly between the ATUs. Our examination of a suite of meristic characters found plate, fin ray, and gill raker counts that were significant between the three ATUs, and several of these counts did not overlap between *S. platorynchus* and *S. albus*. Numerous morphometric characters that differentiated these ATUs were also identified. Some of these measurements have been used extensively in character indices (inner barbel length, snout to outer barbel base, anterior mouth to inner barbel base), others have been used only occasionally (mouth width, orbit length), and several are useful "alternative" measurements (head depth anterior to parietal ridge, dorsal-fin base, anal fin to dorsal fin origin, fifth dorsal plate and spine length, anterior edge of mouth to base of outer barbel and to head edge at outer barbel base, and head width at anterior edge of mouth). Outer barbel length and head length are used extensively in character indices, yet we found no or minimal significant differences between the three ATUs for these measurements. Rostral length and tenth lateral plate height have also been used in indices, but these measurements also showed minimal differences. Multivariate analyses of both meristic and morphometric data provide complete or almost complete separation between ATUs. The use of PCA analyses with these data offers a more powerful tool for differentiating these sturgeon species and their purported hybrids, relative to the resolution provided by traditional character indices.

The ability to correctly identify live specimens of *S. albus* is essential for any fisheries biologist studying this sturgeon or collecting specimens for brood stock. Reliable identification for any of these ATUs is crucial 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 that exists in all current molecular analyses of *Scaphirhynchus*. This problem may explain the continued difficulties in the genetic analyses seeking to identify unique genetic markers for these species and efforts to understand populational and phylogenetic relationships. Recording appropriate data from these released specimens and making it available is essential for researchers to have any scientific or legal basis for genetic or any other studies. Additional data recording via photographs or videotape are also advisable.

Our results indicate that the collection of 6 meristic and 12 morphometric characters, followed by multivariate analyses of these data on the appropriate size classes, can lead to reliable and accurate specimen identification. Because no *a priori* identification of a specimen is required with these analyses, positive field identifications are not as critical, although some form of vouchering (e.g. preserved specimen, photographs, video) is preferred. The 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.

Acknowledgements

Mark Dryer and Steve Krentz, former and current leader of the Pallid Sturgeon Recovery Team (USFWS), served as project officers for this study. Herb Bollig (USFWS) was project leader involved in spawning and raising specimens used in this study. Melissa Kuhajda assisted in data collection. Phil Harris, David Neely, and Steve Powers provided helpful comments and insights during this study. This project was funded by Grant Agreement No. 1448-60181-98-G616 from the USFWS and with funds from the University of Alabama Department of Biological Sciences.

References

Atchley, W. R., C. T. Gaskins, and D. Anderson. 1976. Statistical properties of ratios. I. Empirical results. Systematic Zoology 25:137-148.

Bailey, R. M., and F. B. Cross. 1954. River sturgeon of the American genus *Scaphirhynchus*: Characters, distribution, and synonymy. Papers of the Michigan Academy of Science, Arts, and Letters 39:169-208.

Bookstein, F. L., B. Chernoff, R. L. Elder, J. M. Humphries, Jr., G. R. Smith, and R. E. Strauss. Morphometrics in Evolutionary Biology. The Academy of Natural Sciences of Philadelphia Special Publication 15, Philadelphia, PA.

Campton, D. E., A. L. Bass, F. A. Chapman, and B. W. Bowen. 2000. Genetic distinction of pallid, shovelnose, and Alabama sturgeon: emerging species and the US Endangered Species Act. Conservation Genetics 1:17-32.

Carlson, D. M., and W. L. Pflieger. 1981. Abundance and life history of the lake, pallid, and shovelnose sturgeons in Missouri. Final report, endangered species project SE-1-6, Missouri Department of Conservation, Jefferson City.

Carlson, D. M., W. L. Pflieger, L. Trial, and P. S. Haverland. 1985. Distribution, biology and hybridization of *Scaphirhynchus albus* and *S. platorynchus* in the Missouri and Mississippi rivers. Environmental Biology of Fishes 14:51-59.

Hubbs, C. L., and K. F. Lagler. 1974. Fishes of the Great Lakes region. University of Michigan Press, Ann Arbor.

Humphries, J. M., F. L. Bookstein, B. Chernoff, G. R. Smith, R. L. Elder, and S. G. Poss. 1981. Multivariate discrimination by shape in relation to size. Systematic Zoology 30:291-308.

Jackson, D. A., and K. M. Somers. 1991. The spectre of "spurious" correlation. Oecologia 86:147-151.

Keenlyne, K. D., C. J. Henry, A. Tews, and P. Clancey. 1994. Morphometric comparisons of upper Missouri River sturgeons. Transactions of the American Fisheries Society 123:779-785.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1983. Consistently high meristic counts in natural hybrids between brook trout and bull trout. Systematic Zoology 32(4):369-376.

Mayden, R. L., and B. R. Kuhajda. 1996. Systematics, taxonomy, and conservation status of the endangered Alabama sturgeon, *Scaphirhynchus suttkusi* Williams and Clemmer (Actinopterygii, Acipenseridae). Copeia 1996:241-273.

McQuown, E. C., B. L. Sloss, R. J. Sheehan, J. Rodzen, G. J. Tranah, and B. May. 2000. Microsatellite analysis of genetic variation in sturgeon: new primer sequences for *Scaphirhynchus* and *Acipenser*. Transactions of the American Fisheries Society 129:1380-1388.

Neff, N. A., and G. R. Smith. 1979. Multivariate analysis of hybrid fishes. Systematic Zoology 28:176-196.

Prairie, Y. T., and D. F. Bird. 1989. Some misconceptions about the spurious correlation problem in the ecological literature. Oecologia 81:285-289.

Sheehan, R. J., R. C. Heidinger, P. S. Wills, M. A. Schmidt, G. A. Conover, and K. L. Hurley. 1999. Guide to pallid sturgeon shovelnose sturgeon character index (CI) and morphometric character index (mCI). Southern Illinois University at Carbondale Fisheries Bulletin No. 14.

Simons, A. M., R. M. Wood, L. S. Heath, B. R. Kuhajda, and R. L. Mayden. 2001. Phylogenetics of *Scaphirhynchus* based on mitochondrial DNA sequences. Transactions of the American Fisheries Society.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry, 2nd ed. W. H. Freeman and Co., San Francisco, CA.

U. S. Fish and Wildlife Service. 1993. Pallid sturgeon recovery plan. U. S. Fish and Wildlife Service, Bismarck, ND.

U. S. Fish and Wildlife Service. 2000. Character index for pallid and shovelnose sturgeon. Technical Notes from Missouri River Fish and Wildlife Management Assistance Office 1:96.

Warren, M. J., Jr., B. M. Burr, and B. R. Kuhajda. 1986. Mississippi River sturgeons: new Kentucky records and comments on status. Transactions of the Kentucky Academy of Science 47(1-2):52-53.

Wilde, G. R., and A. A. Echelle. 1997. Morphological variation in intergrade pupfish populations from the Pecos River, Texas, U.S.A. Journal of Fish Biology 50:523-539.

Table 1. Pearson Product-Moment correlation coefficient (r) of significant correlations of size (SL) versus meristic variables for specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, *S. albus*, and all analytical taxonomic units (ATUs). Bold indicates $P < 0.01$; NS refers to not significant.

Meristic Character	*S. platorynchus*	*S. platorynchus* x *S. albus*	*S. albus*	All ATUs
Dorsal plates	NS	NS	0.569	NS
Lateral plates	NS	**-0.493**	NS	**-0.349**
Ventral-lateral plates	NS	NS	**-0.688**	NS
Plates between anus and anal fin	0.641	NS	NS	NS
Plates posterior to anal fin	NS	NS	0.559	NS
Pectoral-fin rays	NS	-0.343	**-0.779**	**-0.427**
Pelvic-fin rays	NS	-0.397	-0.661	-0.293
Gill rakers	**0.775**	**0.556**	NS	**0.530**
Tips of gill rakers	**0.951**	**0.803**	NS	**0.743**

Table 2. Frequency distribution of four meristic characters for small (< 250 mm SL) and large (> 250 mm SL) specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*. A single asterisk indicates character is significantly different between these two or among all three ATUs. A double asterisk denotes an ATU is significantly different from both other ATUs.

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 x *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 x *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**	1			2			1	1		1	1							7	49.4	3.60
S. platorynchus x *S. albus**						1	1	2	2	6	2	7	1		1			22	53.6	1.97
*S. albus**													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**	1	3			1												5	45.4	1.52
S. platorynchus x *S. albus**					1	1	1	2	2	1		3				1	12	52.5	3.09
S. albus										1	1						2	53.5	0.71

Table 2. Continued.

Gill rakers (specimens < 250 mm SL)

	9	10	11	12	13	14	15	n	x	SD
S. platorynchus		1			3	2	1	7	13.1	1.57
S. platorynchus x *S. albus*	1	1	8	6	5	1		22	11.7	1.16
*S. albus***	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**					1		1			2		1	5	16.8	2.77
S. platorynchus x *S. albus**			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 x *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**			1	2	2	5	4.2	0.84
S. platorynchus x *S. albus**		8	4			12	2.3	0.49
S. albus	1	1				2	1.5	0.71

Table 3. Frequency distribution of nine meristic characters for specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*. A single asterisk indicates character is significantly different between these two or among all three ATUs. A double asterisk denotes an ATU is significantly different from both other ATUs.

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 x *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**	3	8	1		12	7.8	0.58
S. platorynchus x *S. albus**	1	16	15	2	34	8.5	0.66
*S. albus**		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**	1		3	3	3	1		1	12	24.3	1.76
S. platorynchus x *S. albus**			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**	2	5	5			12	9.3	0.75
S. platorynchus x *S. albus*	1	12	14	6	1	34	9.8	0.87
*S. albus**		2	6	5	1	14	10.4	0.84

Table 3. Continued.

Ventral plates between anus and anal fin

	3	4	5	n	x	SD
S. platorynchus	4	8		12	3.7	0.49
S. platorynchus x *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	6	6	1		12	7.5	0.52
S. platorynchus x *S. albus*	19	14			34	7.5	0.56
*S. albus***		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**	2	2	2	3	2	1								12	32.3	1.61
S. platorynchus x *S. albus**			1	1	5	7	5	8	4	3				34	36.0	1.77
*S. albus**						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**	1	1	3	4	1	1	1							12	19.8	1.64
S. platorynchus x *S. albus**					1	6	8	9	5	4	1			34	23.8	1.45
*S. albus**									3	4	3	2	2	14	26.7	1.38

Table 3. Continued.

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

Table 4. Factor loadings for principal components analysis of 13 meristic characters for both small (< 250 mm SL) and large (> 250 mm SL) *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*. See Figure 33 for graphic representation.

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

Table 5. Proportional measurements for small (< 250 mm SL) and large (> 250 mm SL) specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*. All measurements (except standard length) are expressed as thousandths of SL. The coefficient of variation (CV) is presented as percent variation.

	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 operculum	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

Table 5. Continued.

	S. platorynchus (continued)							
	< 250 mm SL (n = 7)				> 250 mm SL (n = 5)			
	Min	Max	x	CV	Min	Max	x	CV
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

Table 5. Continued.

	S. platorynchus (continued)							
	< 250 mm SL (n = 7)				> 250 mm SL (n = 5)			
	Min	Max	x	CV	Min	Max	x	CV
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

Table 5. Continued.

	S. platorynchus x *S. albus*							
	< 250 mm SL (n = 22)				> 250 mm SL (n = 12)			
	Min	Max	x	CV	Min	Max	x	CV
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 operculum	209	244	223	4	200	244	206	4
Snout to tip of spine at head end	219	267	241	5	210	267	219	4
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

Table 5. Continued.

	S. platorynchus x *S. albus* (continued)							
	< 250 mm SL (n = 22)				> 250 mm SL (n = 12)			
	Min	Max	x	CV	Min	Max	x	CV
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

Table 5. Continued.

	S. platorynchus x *S. albus* (continued)							
	< 250 mm SL (n = 22)				> 250 mm SL (n = 12)			
	Min	Max	x	CV	Min	Max	x	CV
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

Table 5. Continued.

	S. albus							
	< 250 mm SL (n = 12)				> 250 mm SL (n = 2)			
	Min	Max	x	CV	Min	Max	x	CV
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 operculum	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
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

Table 5. Continued.

	S. albus (continued)							
	< 250 mm SL (n = 12)				> 250 mm SL (n = 2)			
	Min	Max	x	CV	Min	Max	x	CV
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

Table 5. Continued.

	S. albus (continued)							
	< 250 mm SL (n = 12)				> 250 mm SL (n = 2)			
	Min	Max	x	CV	Min	Max	x	CV
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

Table 6. Statistically significant morphometric characters for pairwise comparisons of small (< 250 mm SL) *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus* using SL as denominator (Kruskal-Wallis) or covariate (ANCOVA). Characters significant in all pairwise comparisons are bold.

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 operculum
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 operculum	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	

Table 6. Continued.

S. platorynchus / *S. albus*	*S. platorynchus* / hybrids	*S. albus* / hybrids
		<u>Kruskal-Wallis</u>
		Anal-fin length
		Anterior mouth to snout
		Snout to base of inner barbel
		Snout to base of outer barbel
		<u>ANCOVA</u>
		Head length
		Orbit length

Table 7. Statistically significant morphometric characters for pairwise comparisons of large (> 250 mm SL) *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus* using SL as denominator (Kruskal-Wallis) or covariate (ANCOVA). Characters significant in all pairwise comparisons are bold.

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 operculum	
	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	

Table 8. Factor loadings for sheared principal components analysis of 51 morphometric characters for small (< 250 mm SL) *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*. See Figure 35 for graphic representation.

Morphometric Character	Loading		
	Size	SPC2	SPC3
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 operculum	-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 t 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

Table 8. Continued.

Measurement	Loading		
	Size	SPC2	SPC3
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

Table 9. Factor loadings for sheared principal components analysis of 51 morphometric characters for large (> 250 mm SL) *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*. See Figure 36 for graphic representation.

	Loading		
Morphometric Characters	Size	SPC2	SPC3
Standard length			
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 operculum	-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 t 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

Table 9. Continued.

Measurement	Loading		
	Size	SPC2	SPC3
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

Table 10. Frequency distribution of spines and spine fusion on head region for small (< 250 mm SL) specimens of *Scaphirhynchus platorynchus* (n = 7), *S. platorynchus* x *S. albus* (n = 22), and *S. albus* (n = 12). For all head spines, the following codes are used: 0 = spine absent, 1 = present but completely fused, forming a ridge, 2 = present and partially fused into ridge, 3 = present and exposed.

	Left preorbital spine				Right preorbital spine			
	0	1	2	3	0	1	2	3
S. platorynchus		2	2	3		1	3	3
S. platorynchus x *S. albus*	2	6	6	8	2	5	5	10
S. albus	3	6	3		1	7	4	
	Left parietal spine				**Right parietal spine**			
	0	1	2	3	0	1	2	3
S. platorynchus		2	4	1		2	5	
S. platorynchus x *S. albus*		12	10			11	10	1
S. albus		5	7			7	5	
	Left posttemporal spine				**Right posttemporal spine**			
	0	1	2	3	0	1	2	3
S. platorynchus	1	2	2	2	2	3	2	
S. platorynchus x *S. albus*	5	6	8	3	2	9	5	6
S. albus		1	10	1		1	7	4
	Left tabular spine				**Right tabular spine**			
	0	1	2	3	0	1	2	3
S. platorynchus			4	3			3	4
S. platorynchus x *S. albus*		4	6	12		1	9	12
S. albus			5	7			2	10

Table 11. Frequency distribution of spines and spine fusion on head region for large (> 250 mm SL) specimens of *Scaphirhynchus platorynchus* (n = 5), *S. platorynchus* x *S. albus* (n = 12), and *S. albus* (n = 2). For all head spines, the following codes are used: 0 = spine absent, 1 = present but completely fused, forming a ridge, 2 = present and partially fused into ridge, 3 = present and exposed.

	Left preorbital spine				Right preorbital spine			
	0	1	2	3	0	1	2	3
S. platorynchus	1	4			1	2	2	
S. platorynchus x *S. albus*		7	5			8	3	1
S. albus		2			1	1		
	Left parietal spine				**Right parietal spine**			
	0	1	2	3	0	1	2	3
S. platorynchus	1	4				5		
S. platorynchus x *S. albus*		12			1	11		
S. albus		2				2		
	Left posttemporal spine				**Right posttemporal spine**			
	0	1	2	3	0	1	2	3
S. platorynchus		5				4	1	
S. platorynchus x *S. albus*	4	8			5	6	1	
S. albus		2				2		
	Left tabular spine				**Right tabular spine**			
	0	1	2	3	0	1	2	3
S. platorynchus		3	2			3	1	1
S. platorynchus x *S. albus*		3	4	5		4	3	5
S. albus		2				2		

Table 12. Proportional measurements of head spines for small (< 250 mm SL) and large (> 250 mm SL) specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*. All measurements are expressed as thousandths of standard length.

	S. platorynchus					*S. platorynchus* x *S. albus*					*S. albus*				
Character	n	Min.	Max.	x	SD	n	Min.	Max.	x	SD	n	Min.	Max.	x	SD
< 250 mm SL															
Preorbital 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
Posttemporal 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

Table 12. Continued.

	S. platorynchus					*S. platorynchus* x *S. albus*					*S. albus*				
Character	n	Min.	Max.	x	SD	n	Min.	Max.	x	SD	n	Min.	Max.	x	SD
> 250 mm SL															
Preorbital 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
Posttemporal 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

Table 13. Statistically significant mensural spine characters for pairwise comparisons of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus* using SL as denominator (Kruskal-Wallis) or covariate (ANCOVA).

S. platorynchus / *S. albus*	*S. platorynchus* / hybrids	*S. albus* / hybrids
Small (< 250 mm SL)		
Kruskal-Wallis / ANCOVA		Kruskal-Wallis / ANCOVA
Preorbital spine right		Preorbital spine right
Kruskal-Wallis		Kruskal-Wallis
Preorbital spine left		Preorbital spine left
Posttemporal spine left		
Large (> 250 mm SL)		
ANCOVA	ANCOVA	ANCOVA
Posttemporal spine left	Parietal spine right	Parietal spine right
Posttemporal spine right	Posttemporal spine right	
Tabular spine right		

Table 14. Position of outer barbel relative to inner barbel for all specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

	Outer barbel relative to inner barbel		
	anterior	even	posterior
S. platorynchus	7	6	
S. platorynchus x *S. albus*		10	24
S. albus			14

Table 15. Number of spines at the posterior-lateral end of head for all specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. 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 x *S. albus*	26	8	22	12
S. albus	10	4	10	4

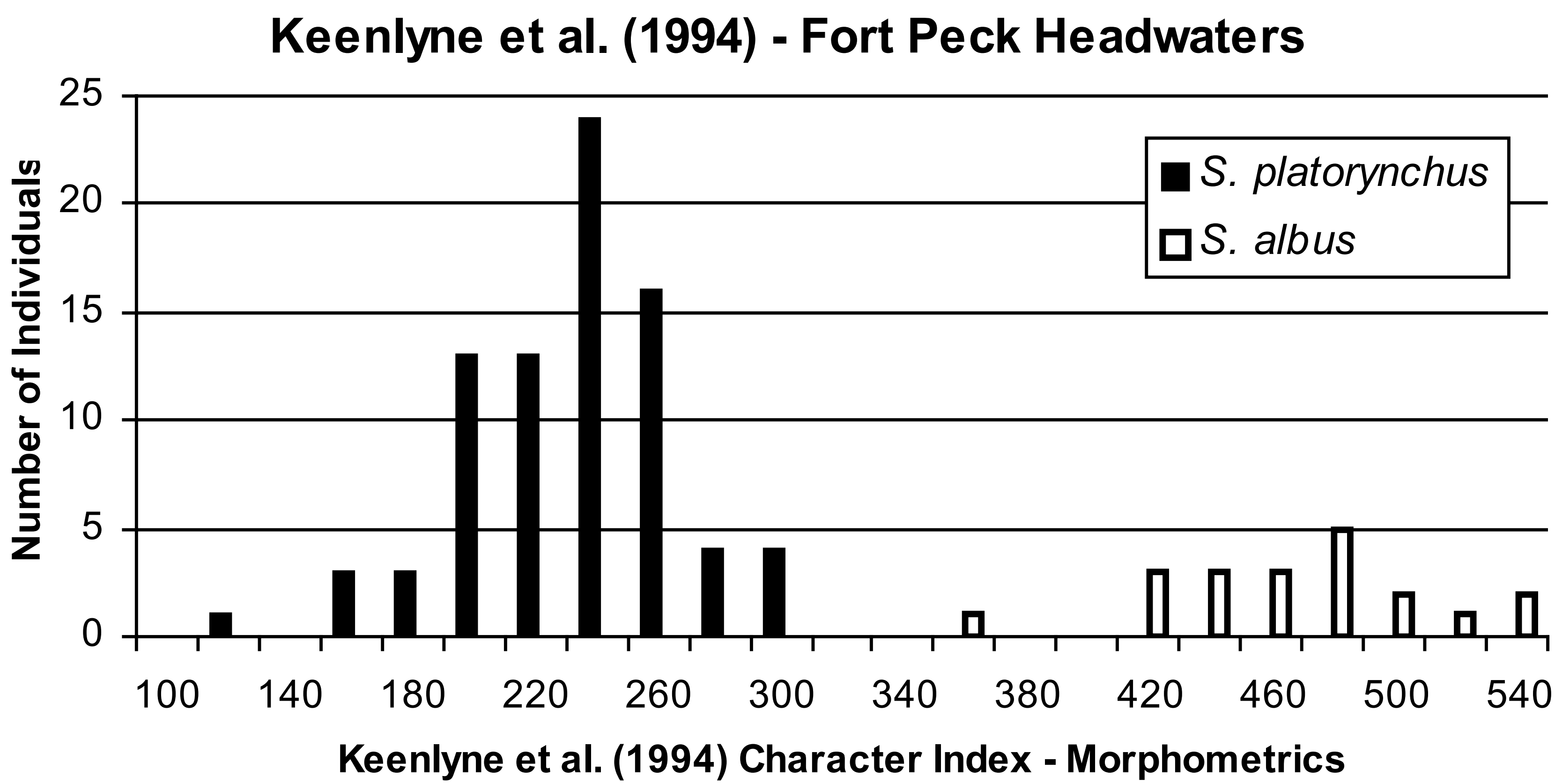

Figure 1. Character index (Keenlyne et al. 1994) based on morphometric characters for specimens of *Scaphirhynchus platorynchus* and *S. albus* collected in the Missouri River from headwaters above Fort Peck Reservoir, Montana.

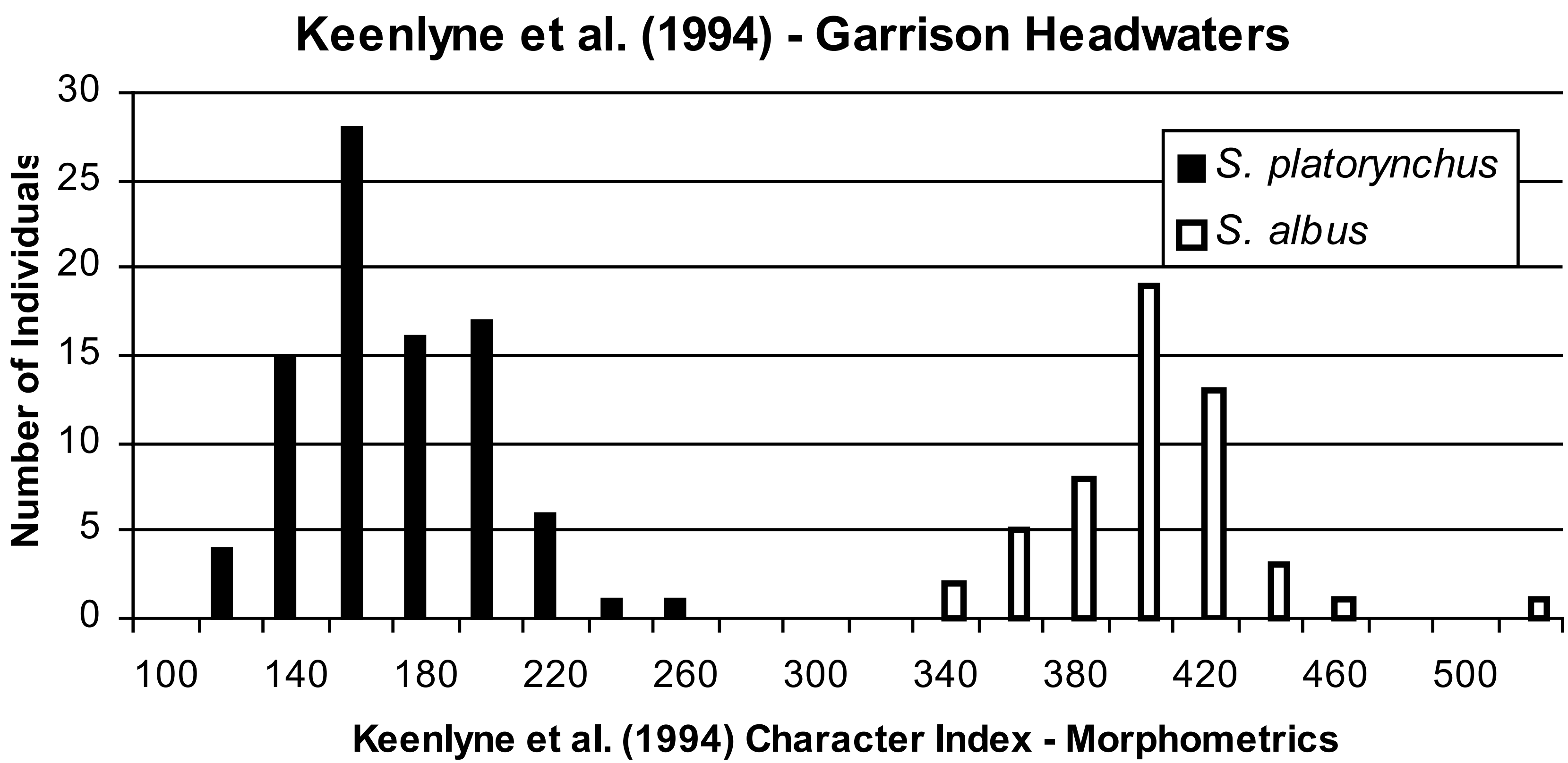

Figure 2. Character index (Keenlyne et al. 1994) based on morphometric characters for specimens of *Scaphirhynchus platorynchus* and *S. albus* collected in the Missouri River from headwaters of Garrison Reservoir, North Dakota.

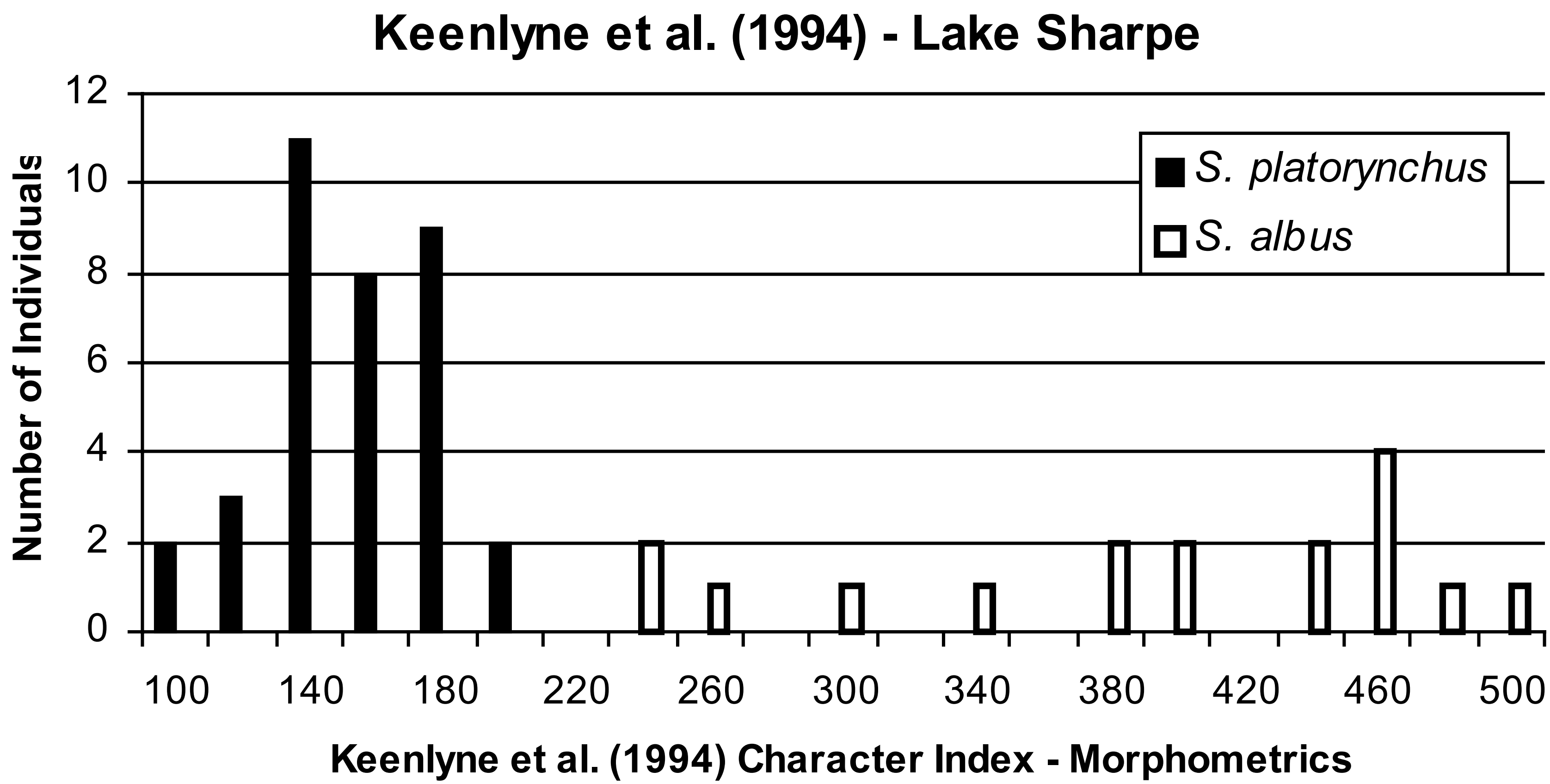

Figure 3. Character index (Keenlyne et al. 1994) based on morphometric characters for specimens of *Scaphirhynchus platorynchus* and *S. albus* collected in the Missouri River from Lake Sharpe, South Dakota.

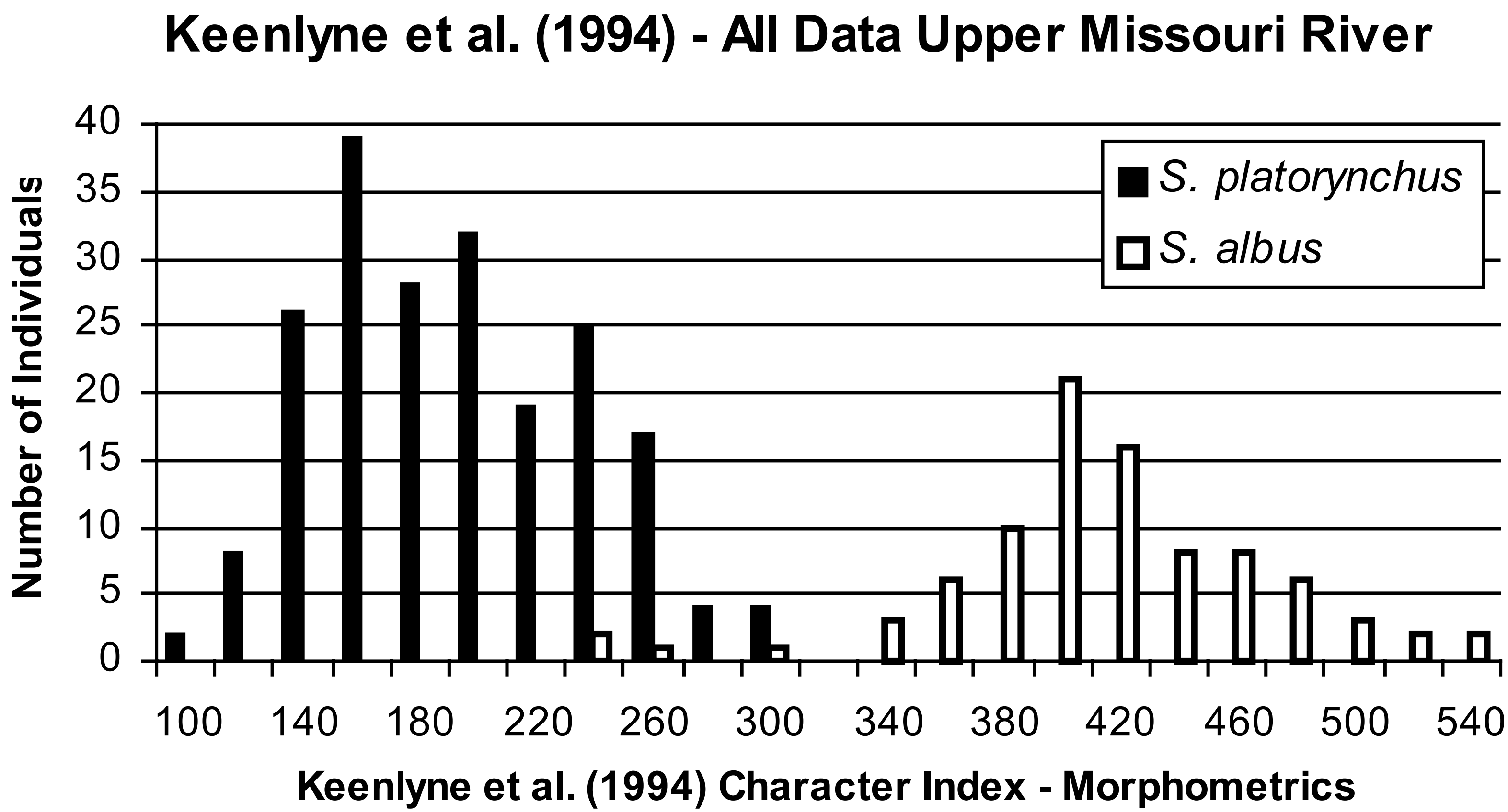

Figure 4. Character index (Keenlyne et al. 1994) based on morphometric characters for combined specimens of *Scaphirhynchus platorynchus* and *S. albus* collected in the Missouri River from all three reservoirs in Montana and North and South Dakota.

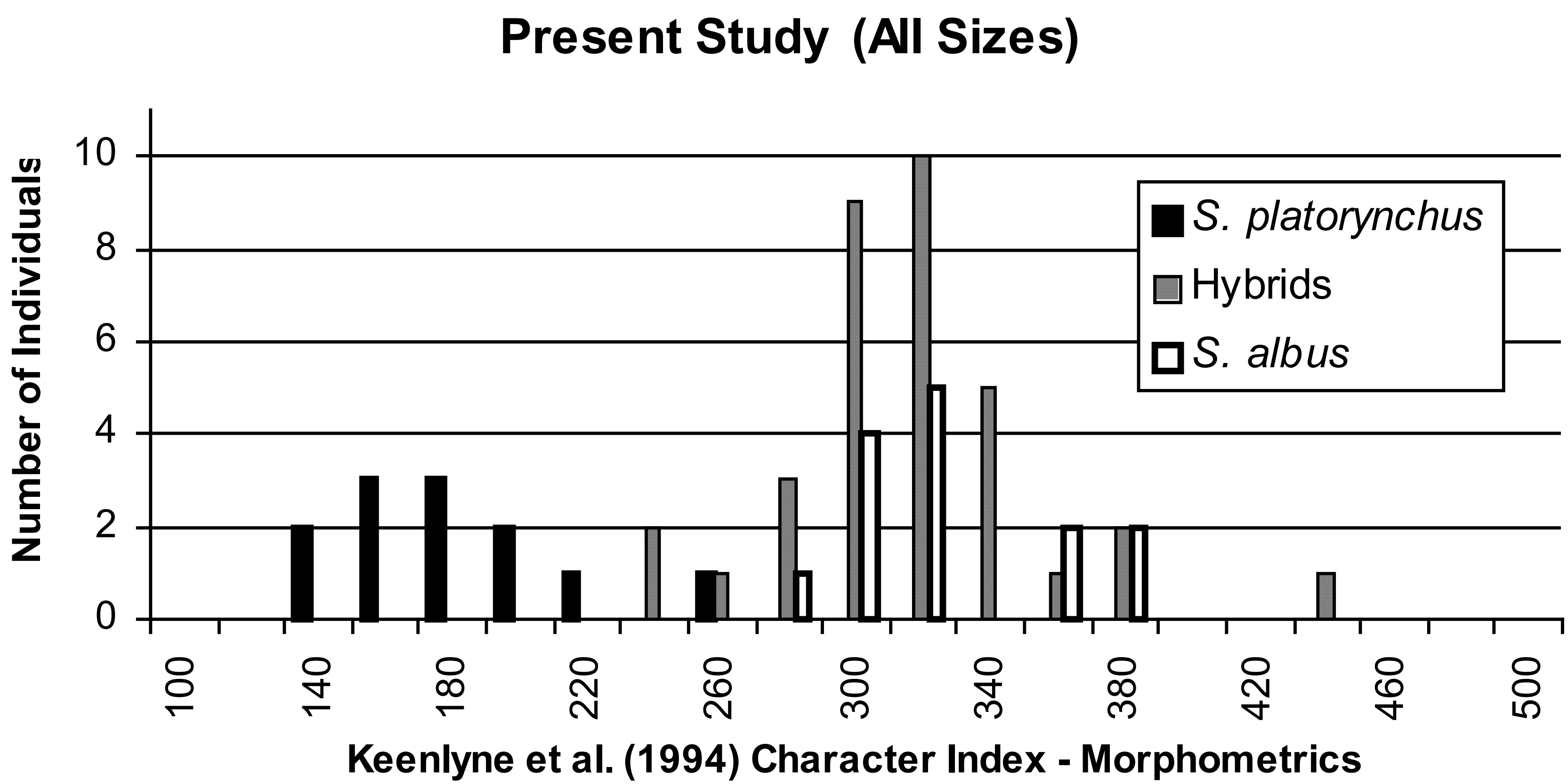

Figure 5. Character index (Keenlyne et al. 1994) based on morphometric characters for all sizes of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

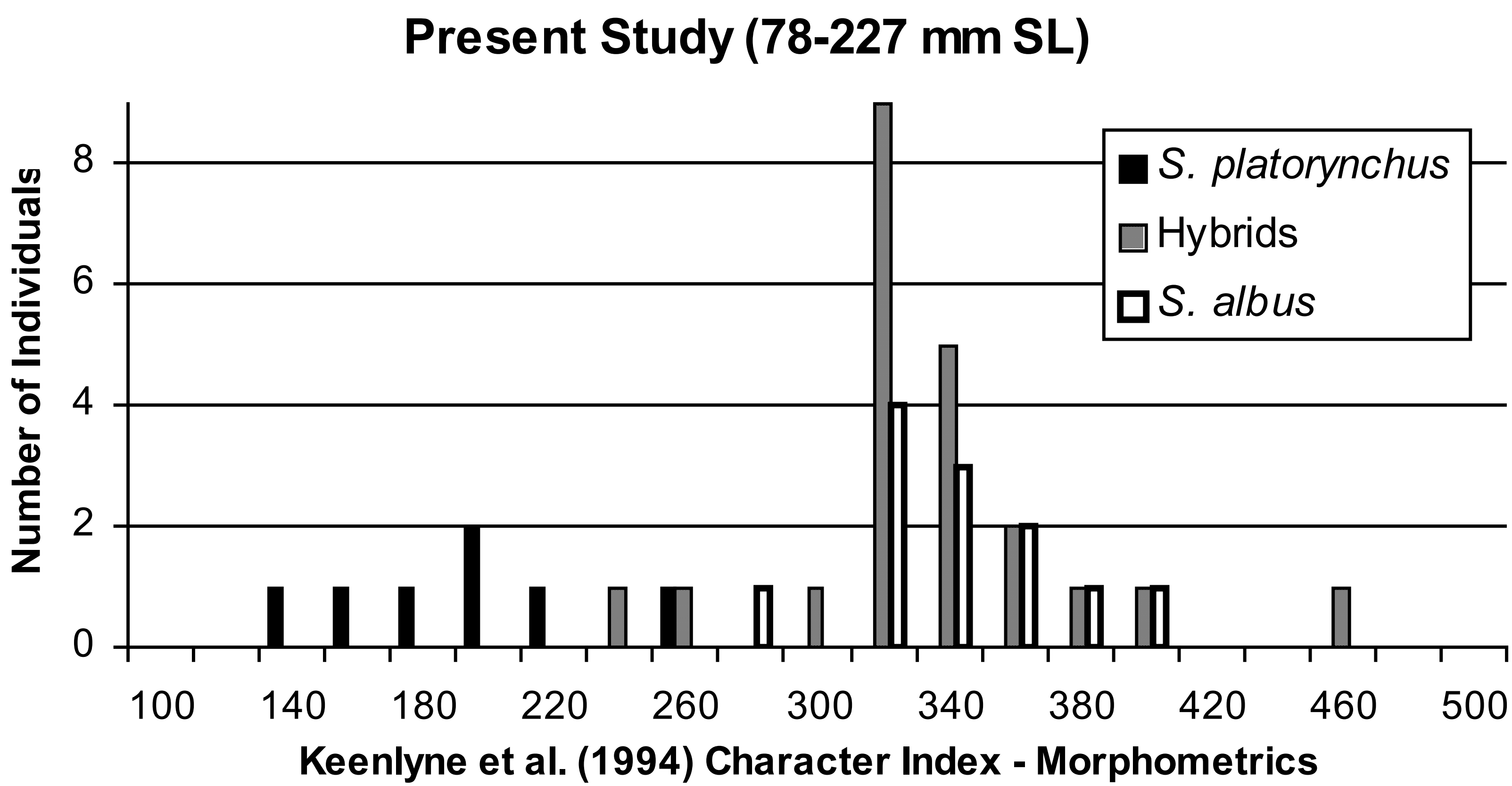

Figure 6. Character index (Keenlyne et al. 1994) based on morphometric characters for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

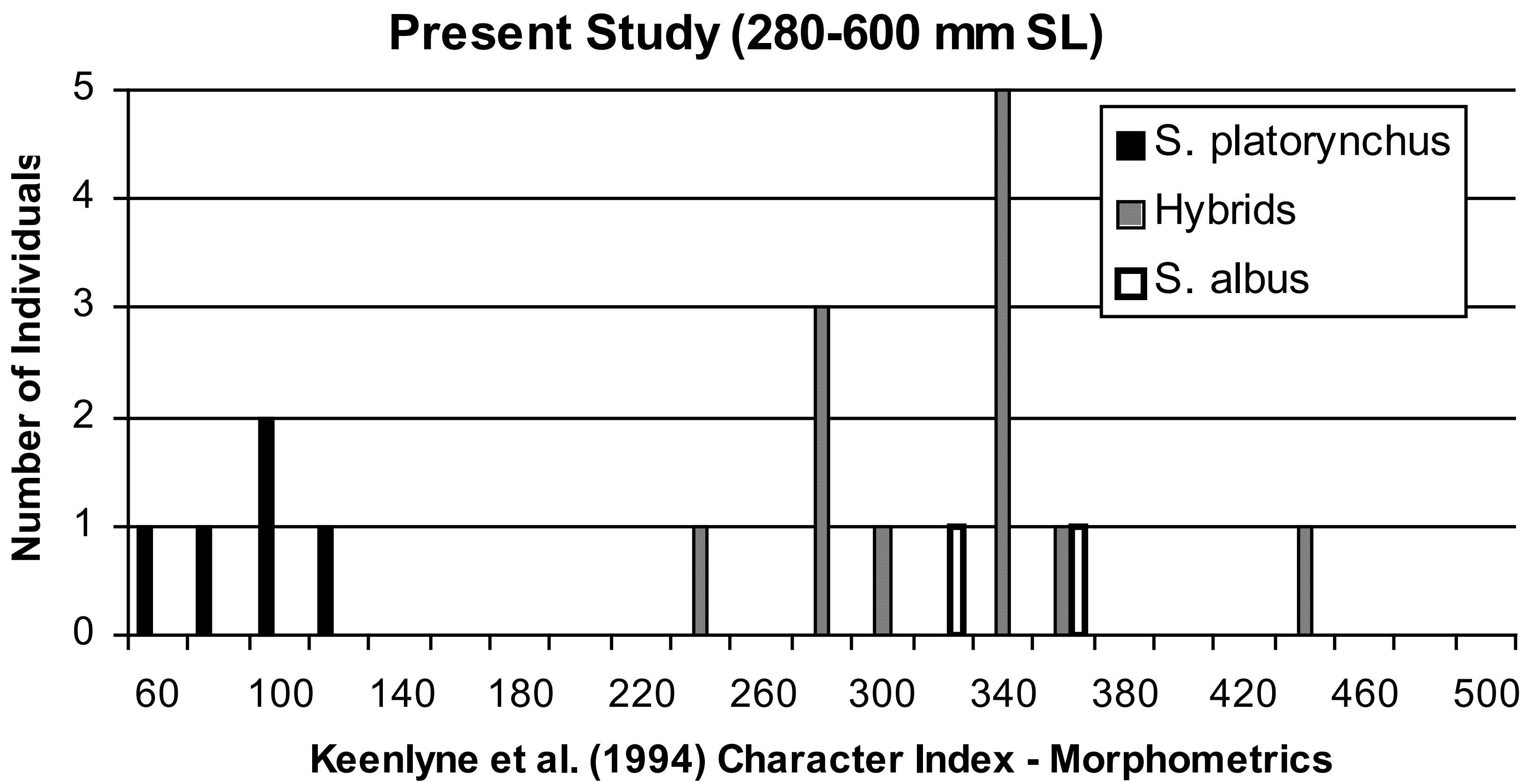

Figure 7. Character index (Keenlyne et al. 1994) based on morphometric characters for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

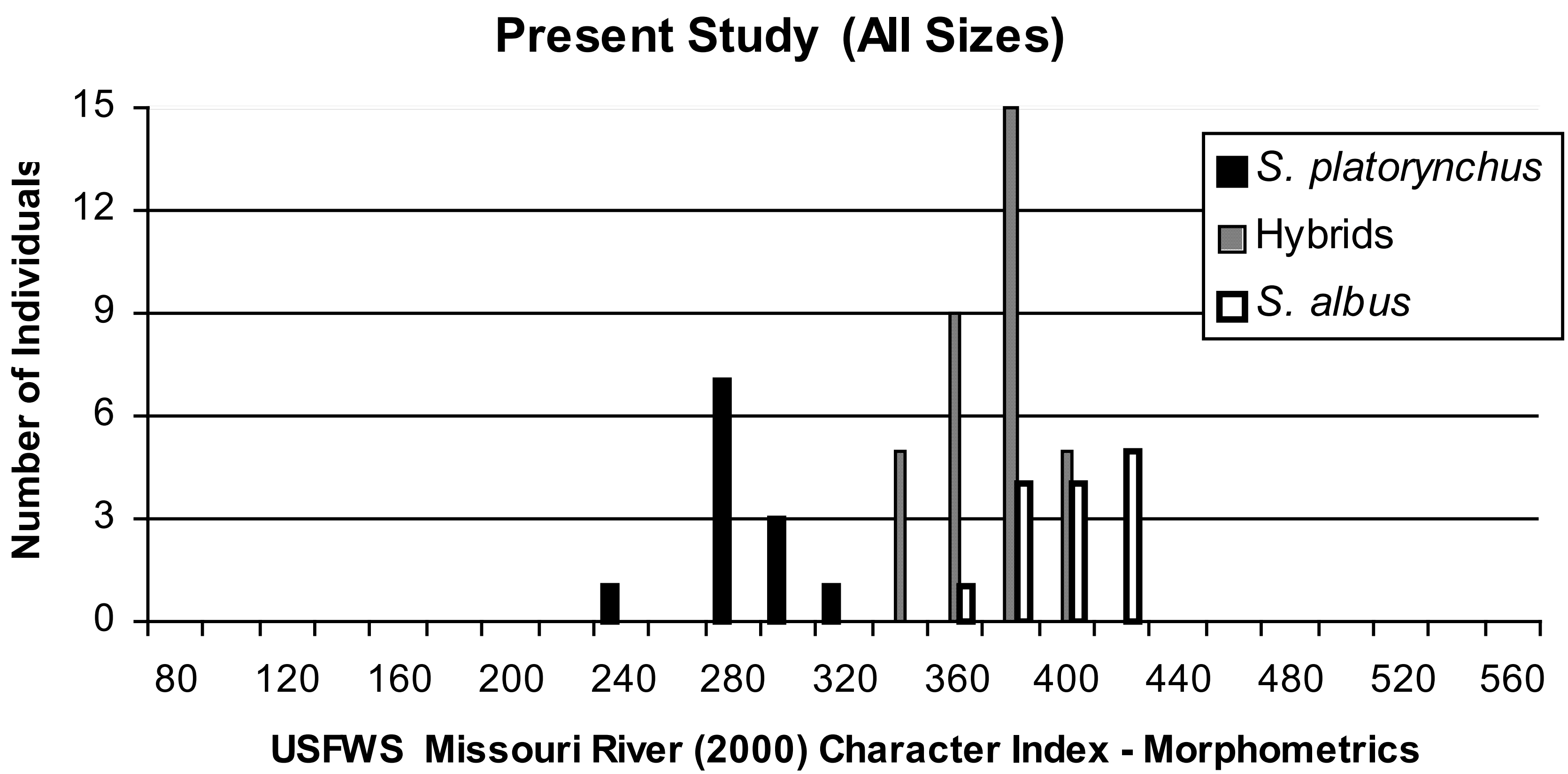

Figure 8. Character index (U.S. Fish and Wildlife Service 2000) based on morphometric characters and minimum and maximum values provided with index for all sizes of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

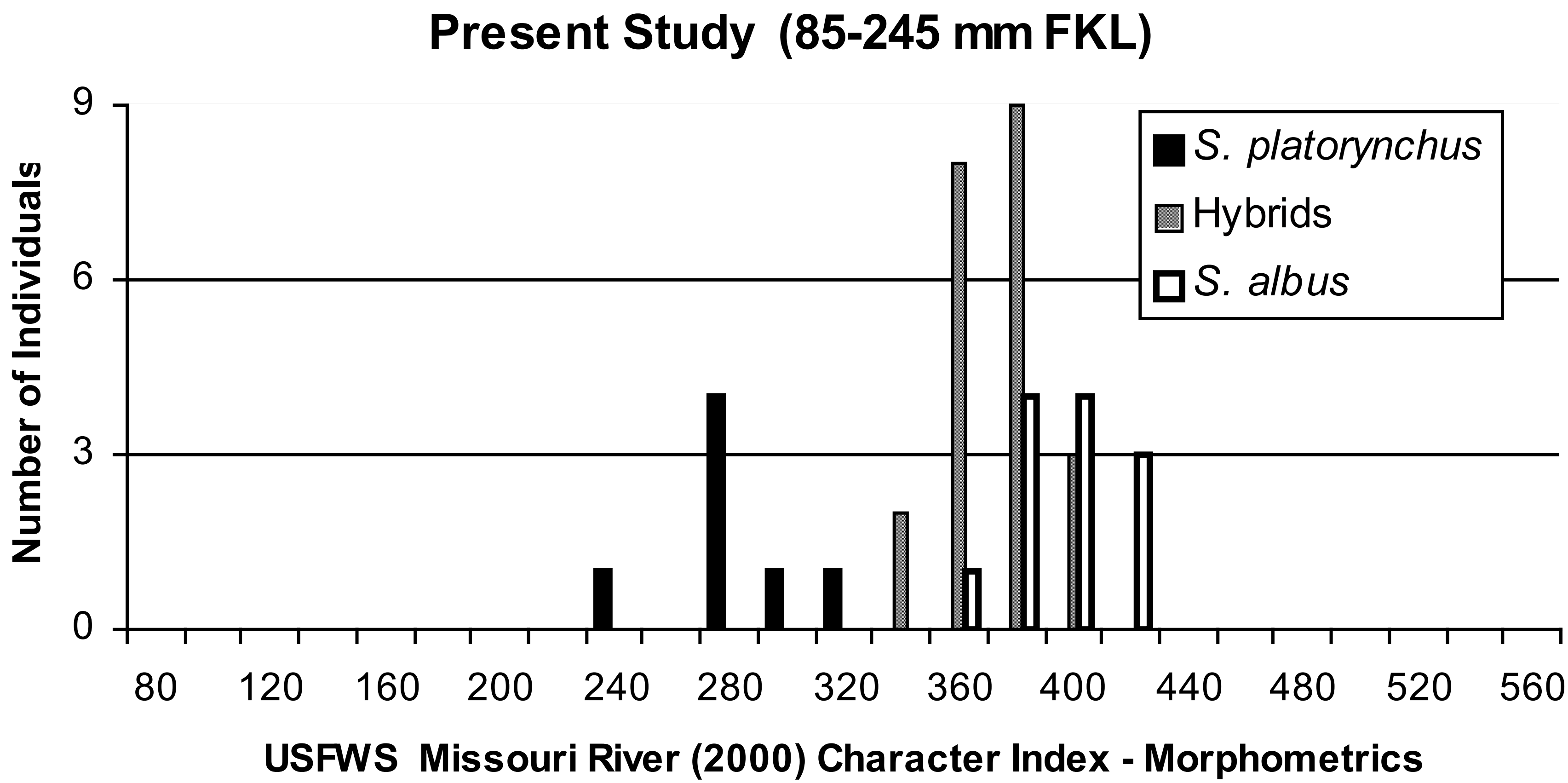

Figure 9. Character index (U.S. Fish and Wildlife Service 2000) based on morphometric characters and minimum and maximum values provided with index for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

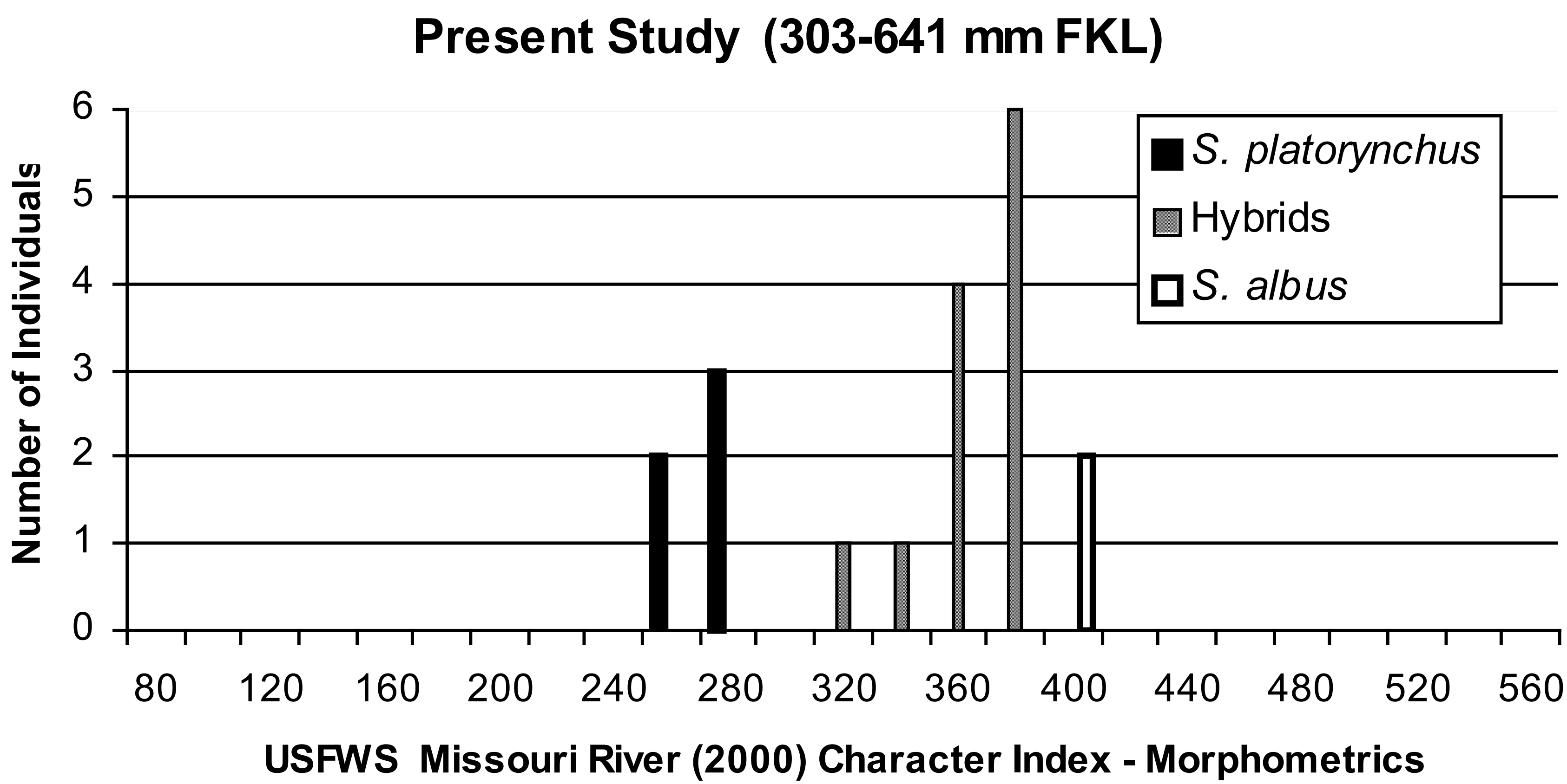

Figure 10. Character index (U.S. Fish and Wildlife Service 2000) based on morphometric characters and minimum and maximum values provided with index for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

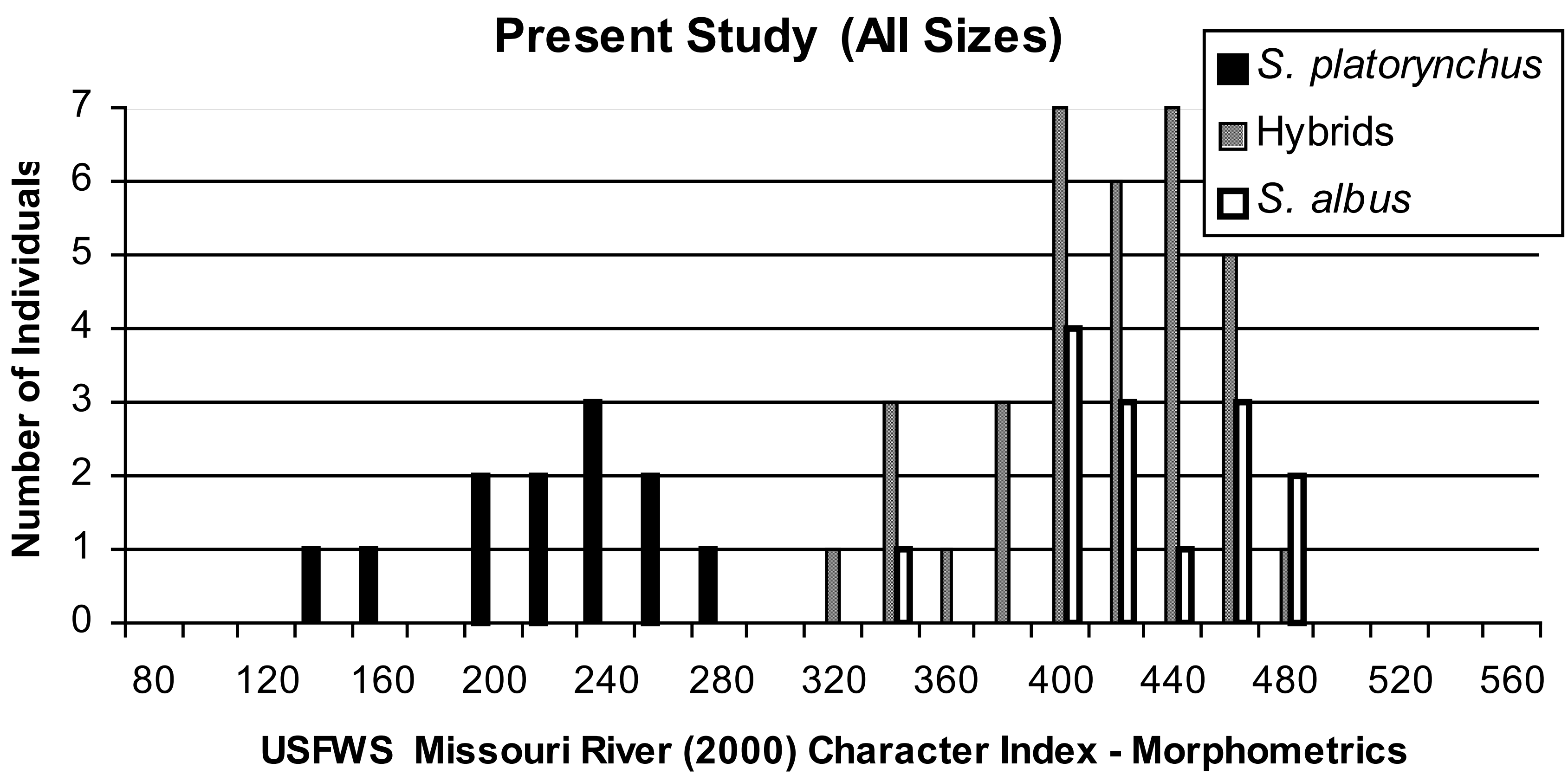

Figure 11. Character index (U.S. Fish and Wildlife Service 2000) based on morphometric characters and minimum and maximum values present in the data for all sizes of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

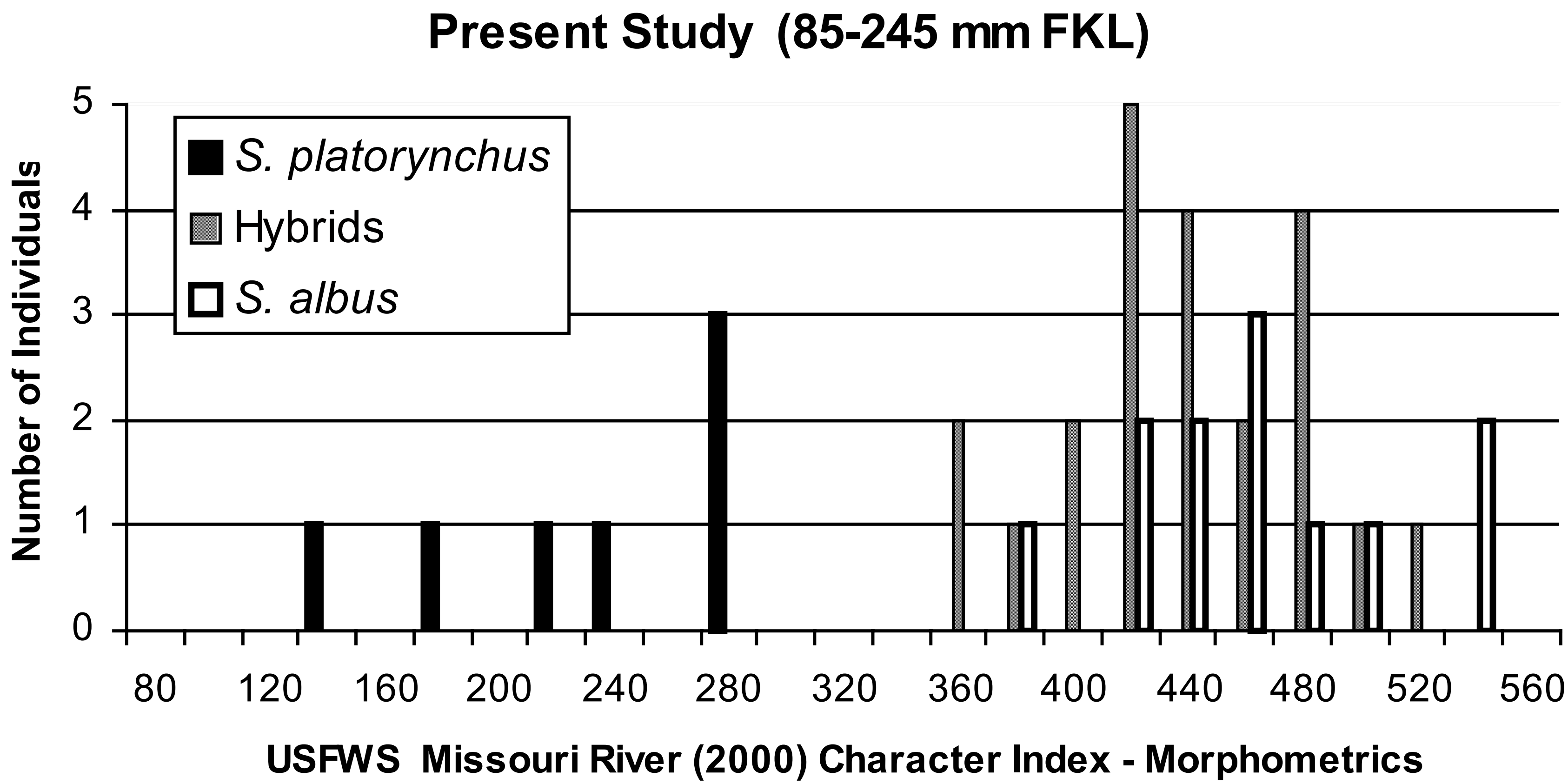

Figure 12. Character index (U.S. Fish and Wildlife Service 2000) based on morphometric characters and minimum and maximum values present in the data for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

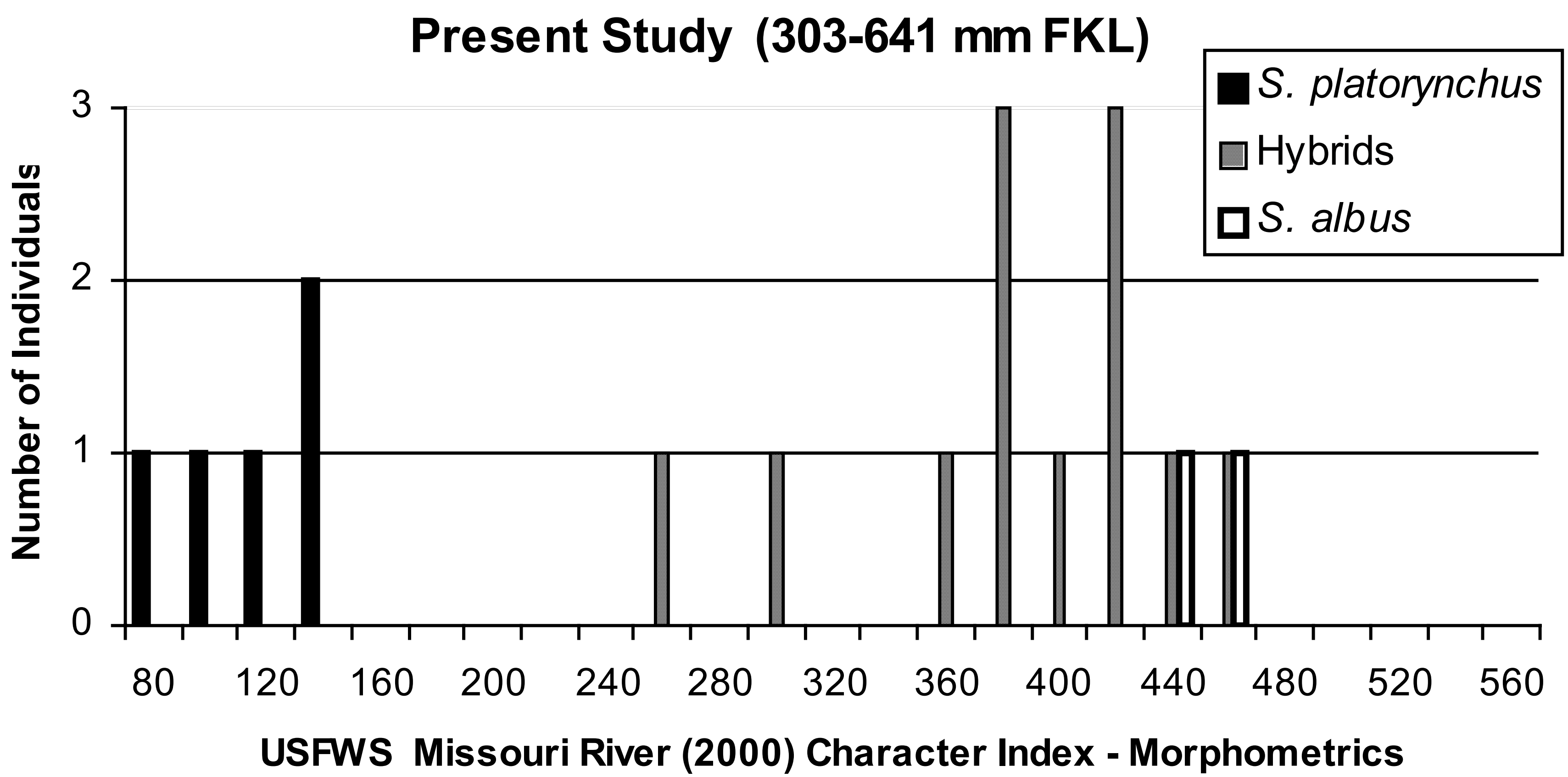

Figure 13. Character index (U.S. Fish and Wildlife Service 2000) based on morphometric characters and minimum and maximum values present in the data for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

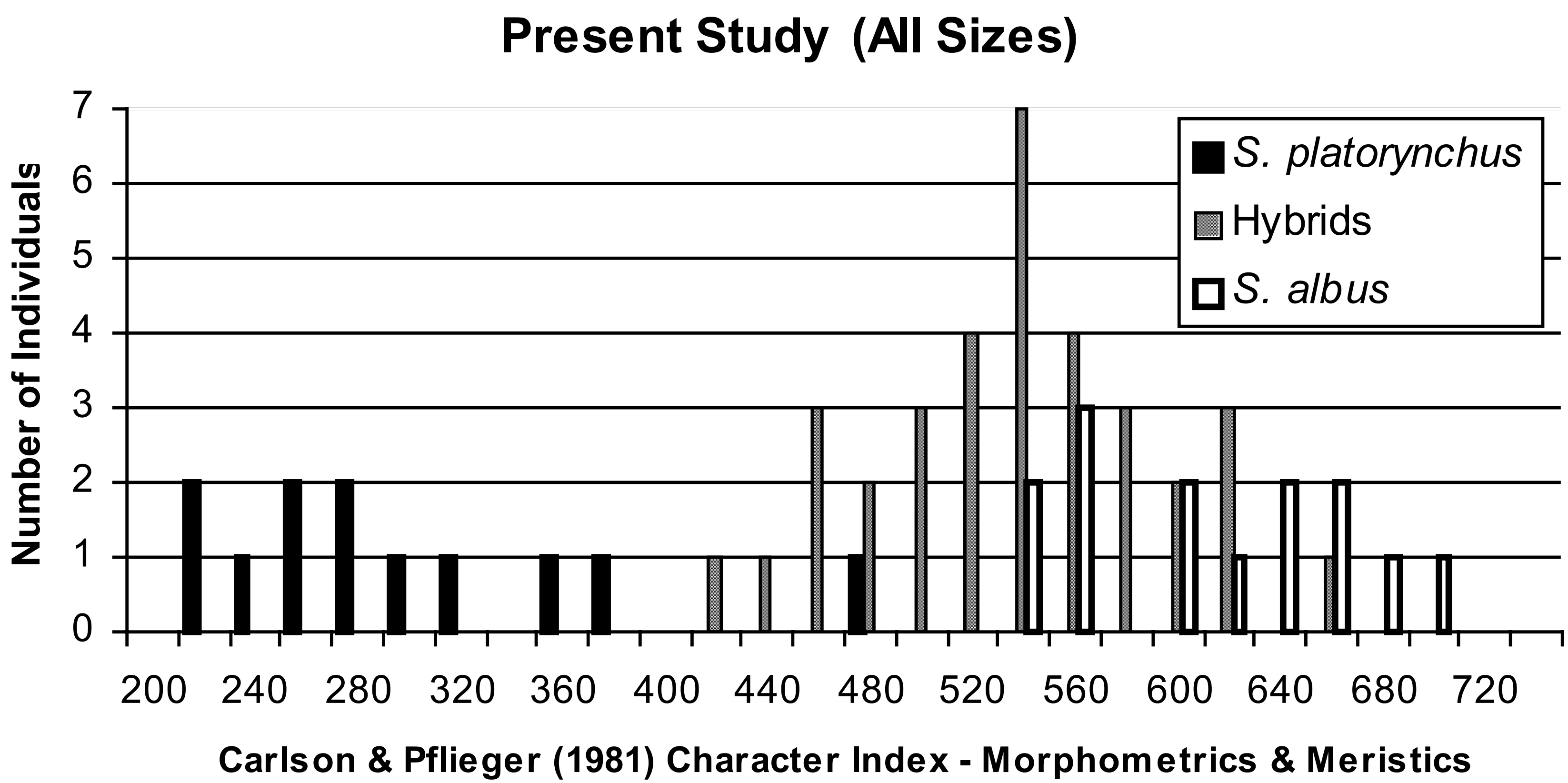

Figure 14. Character index (Carlson and Pflieger 1981) based on morphometric and meristic characters for all sizes of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

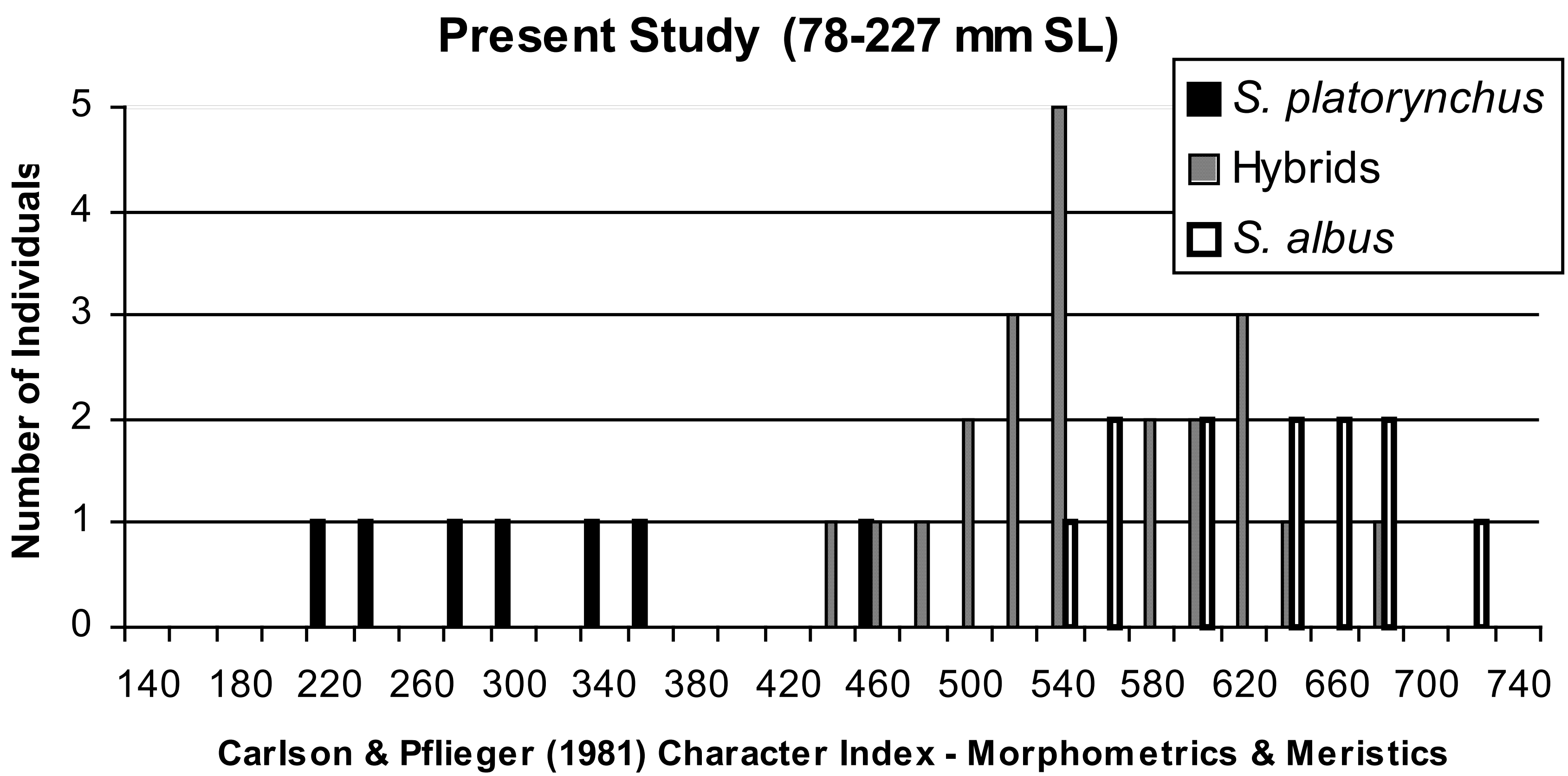

Figure 15. Character index (Carlson and Pflieger 1981) based on morphometric and meristic characters for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

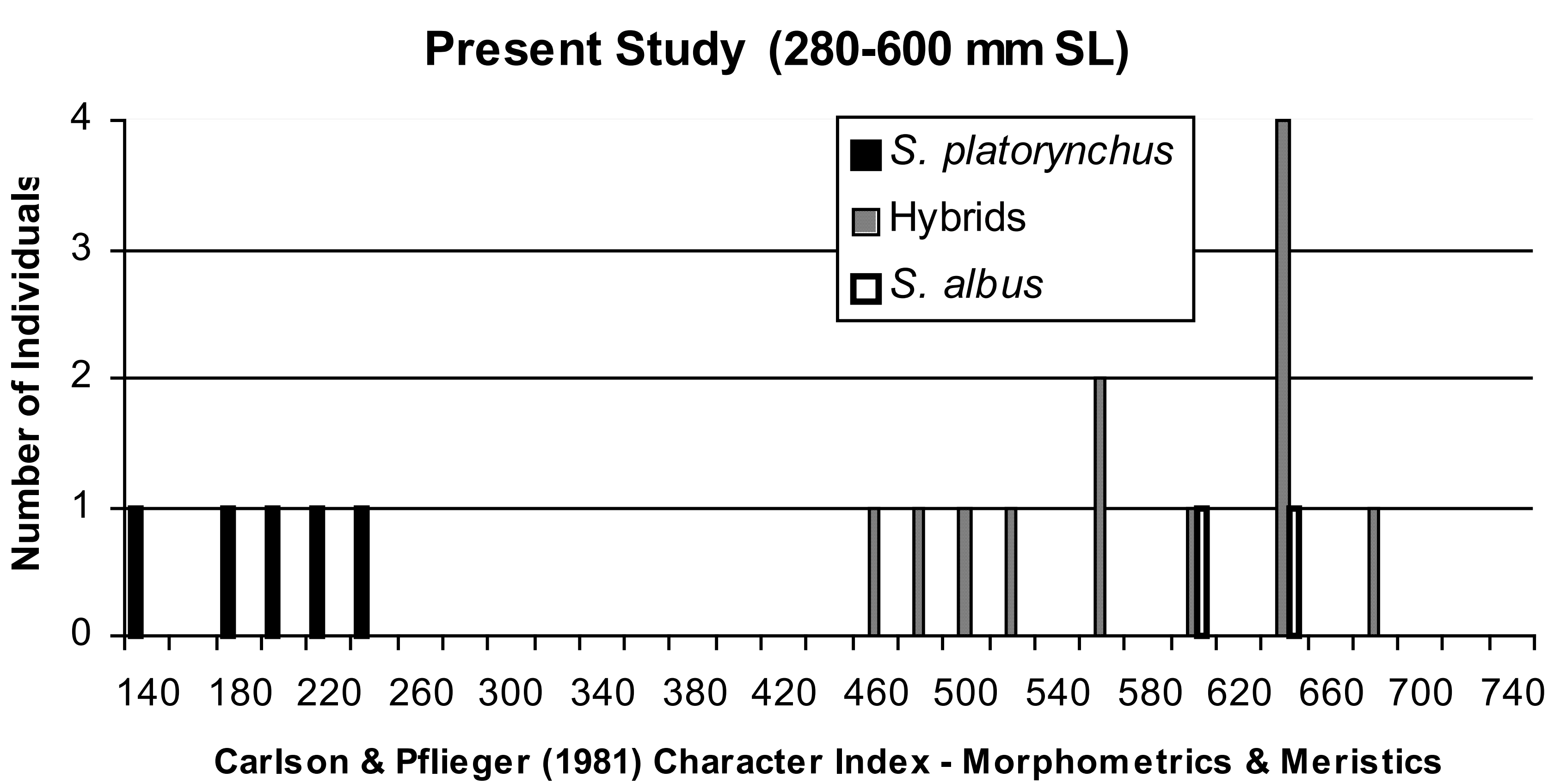

Figure 16. Character index (Carlson and Pflieger 1981) based on morphometric and meristic characters for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

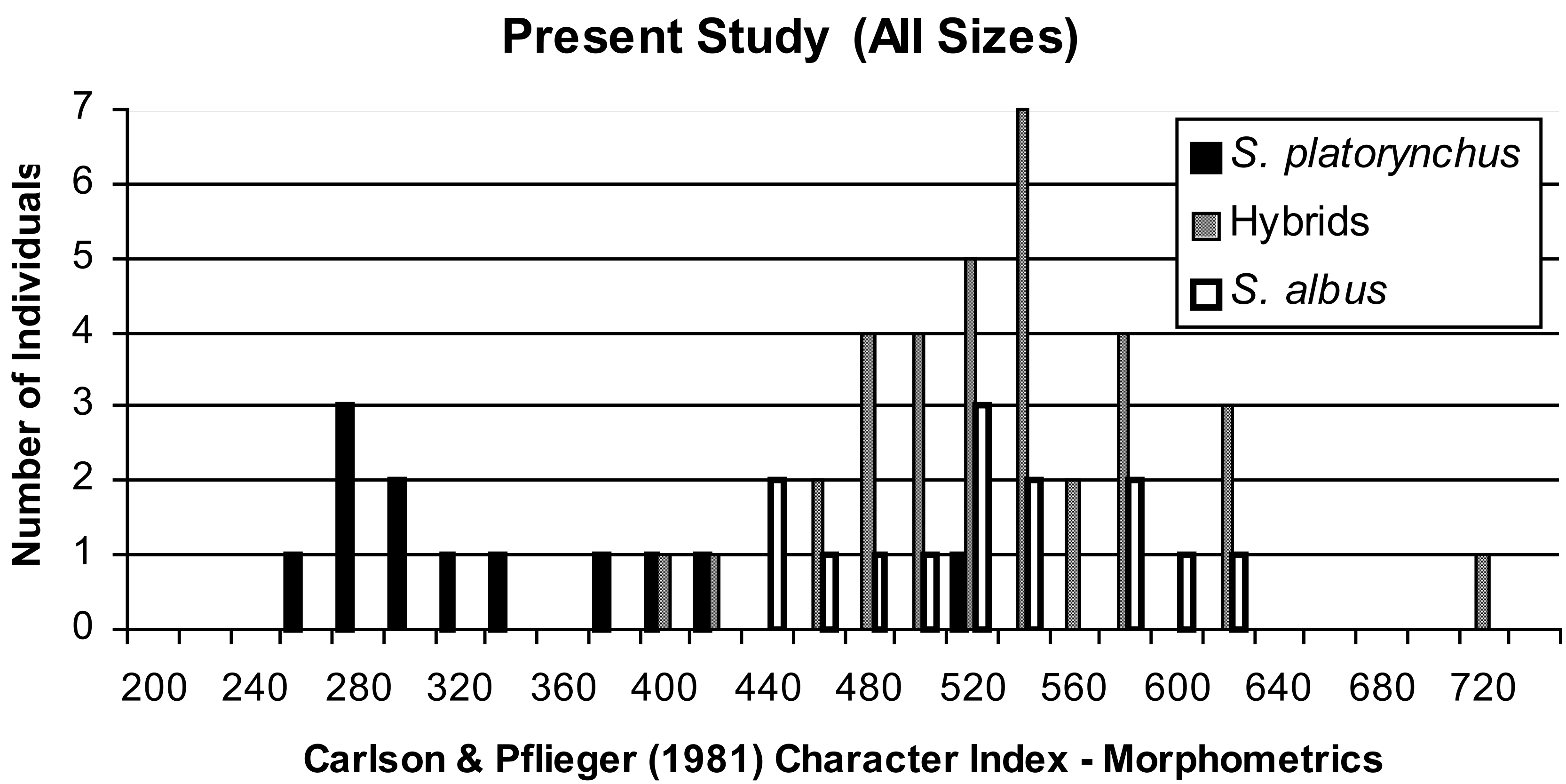

Figure 17. Character index (Carlson and Pflieger 1981) based on morphometric characters for all sizes of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

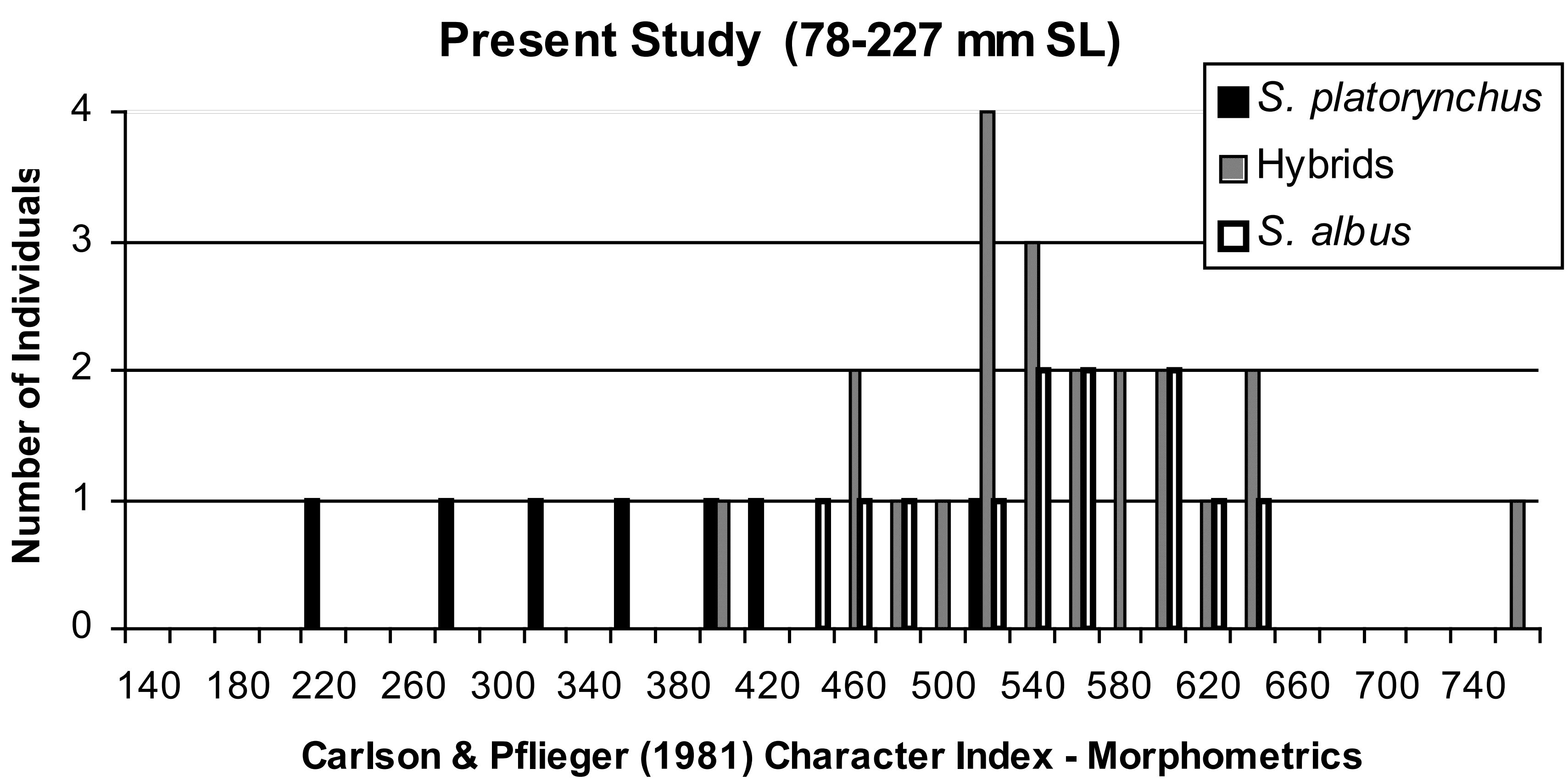

Figure 18. Character index (Carlson and Pflieger 1981) based on morphometric characters for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

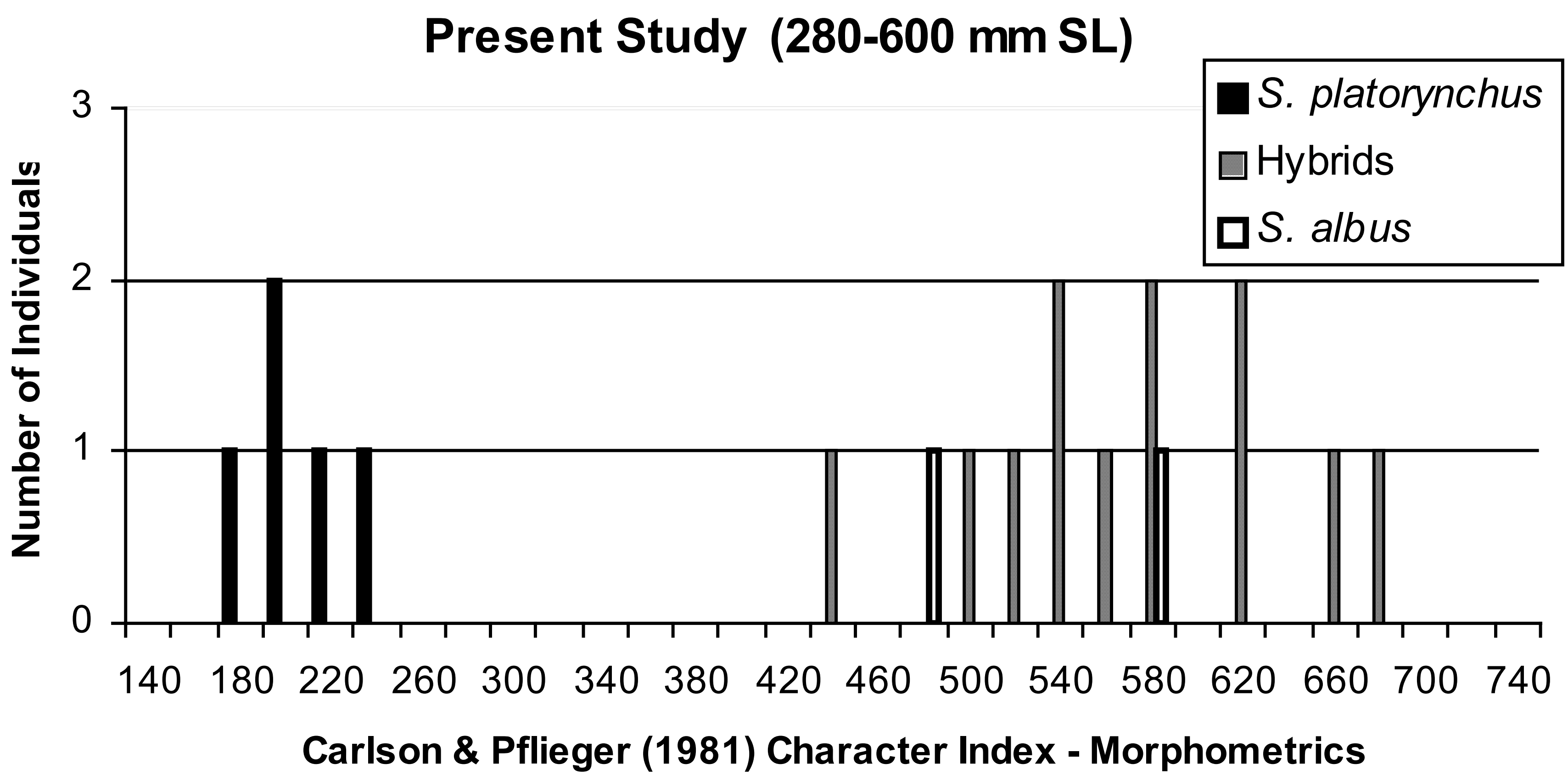

Figure 19. Character index (Carlson and Pflieger 1981) based on morphometric characters for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

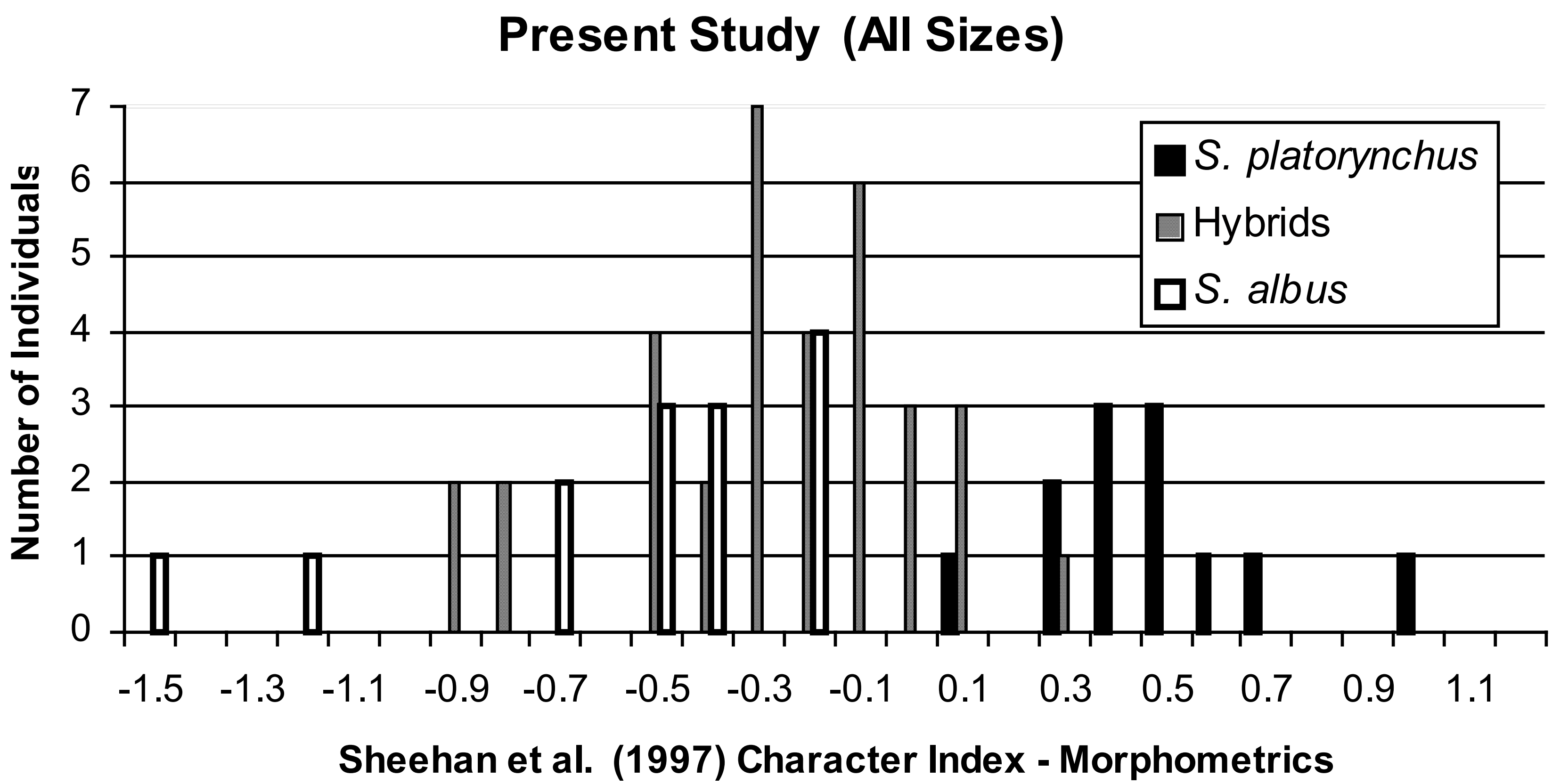

Figure 20. Character index (Sheehan et al. 1997) based on morphometric characters for all sizes of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

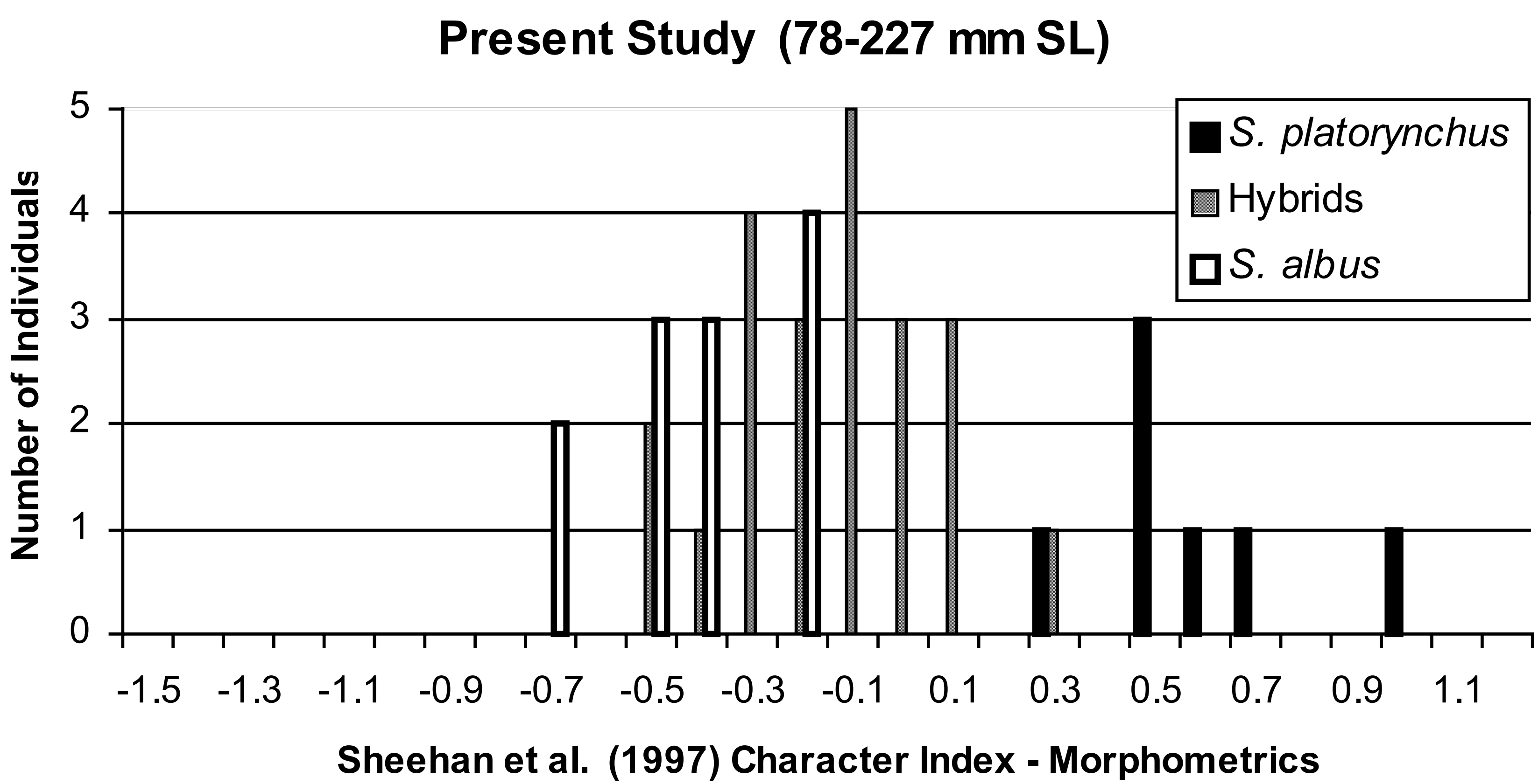

Figure 21. Character index (Sheehan et al. 1997) based on morphometric characters for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

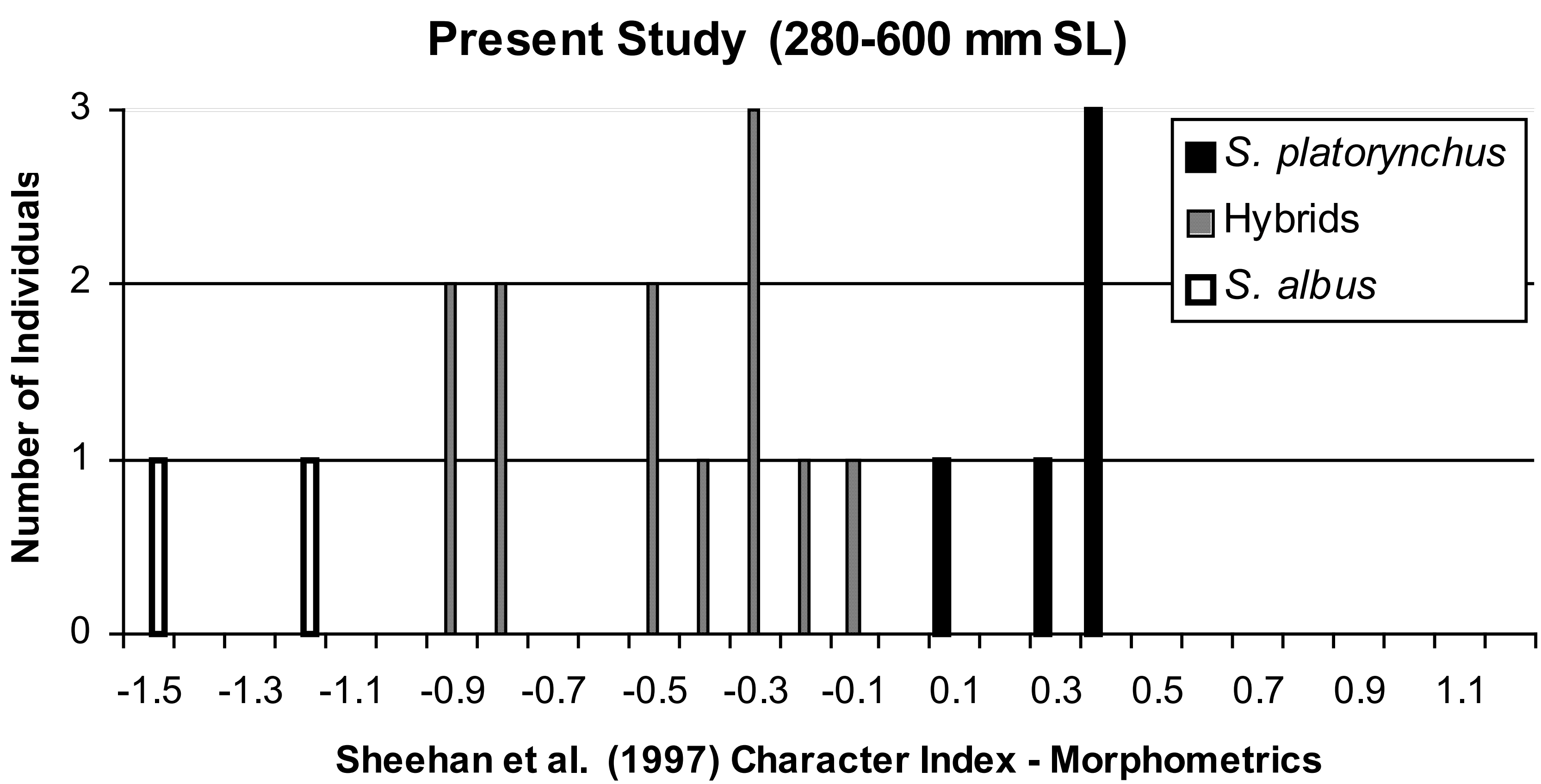

Figure 22. Character index (Sheehan et al. 1997) based on morphometric characters for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

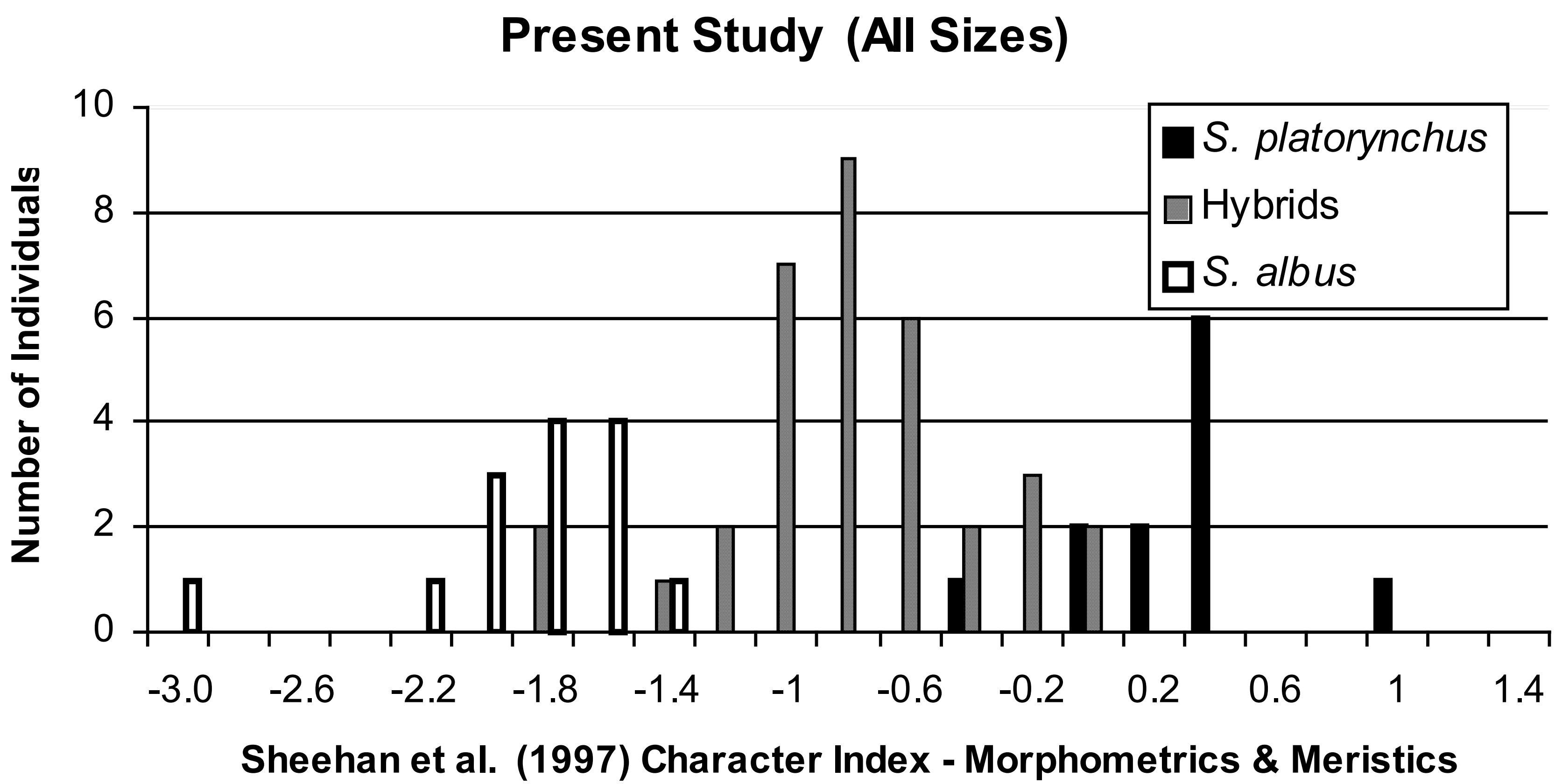

Figure 23. Character index (Sheehan et al. 1997) based on morphometric and meristic characters for all sizes of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

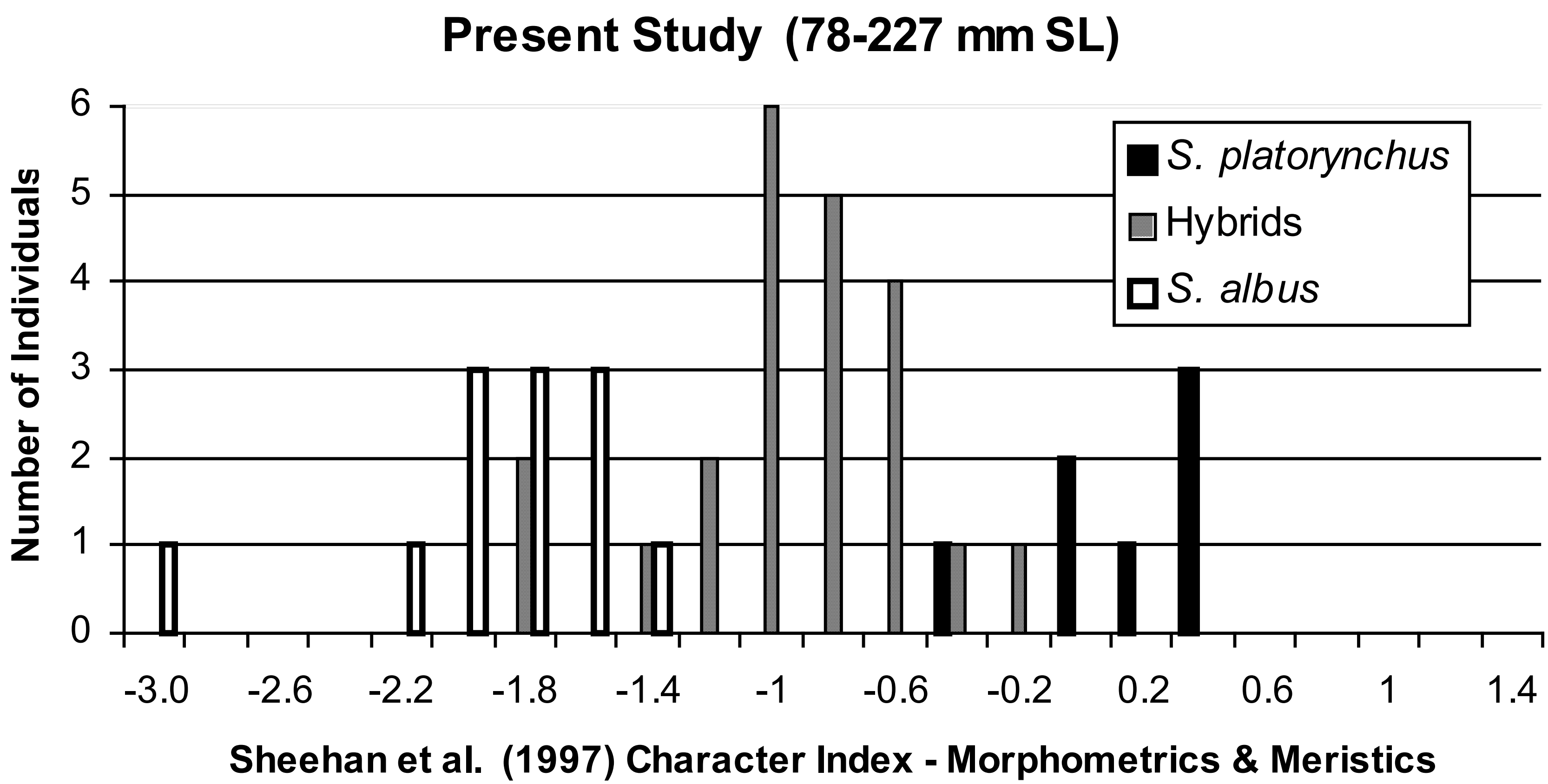

Figure 24. Character index (Sheehan et al. 1997) based on morphometric characters for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

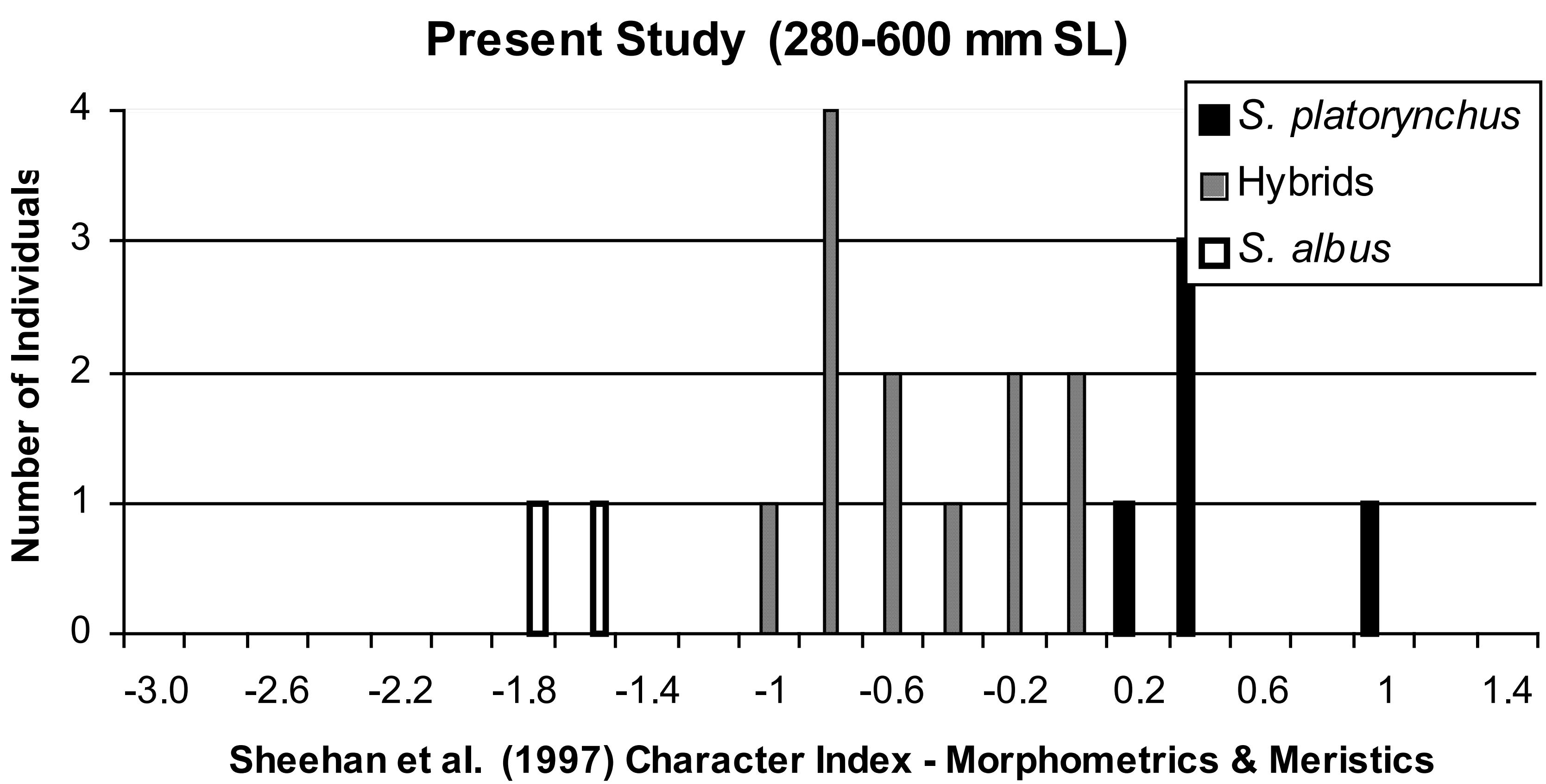

Figure 25. Character index (Sheehan et al. 1997) based on morphometric characters for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

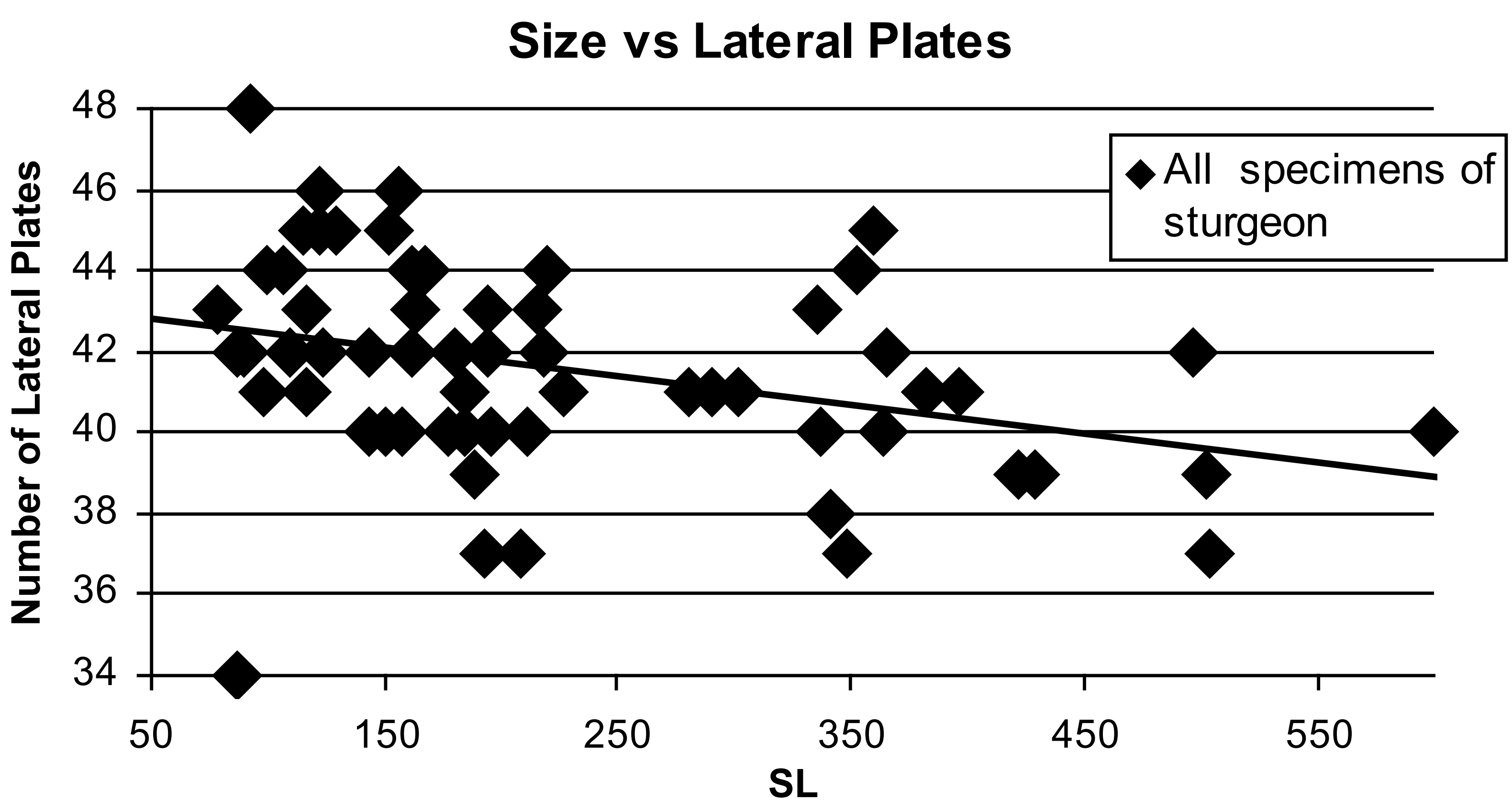

Figure 26. Significant relationship of lateral plates with size for all hatchery-reared *Scaphirhynchus* specimens.

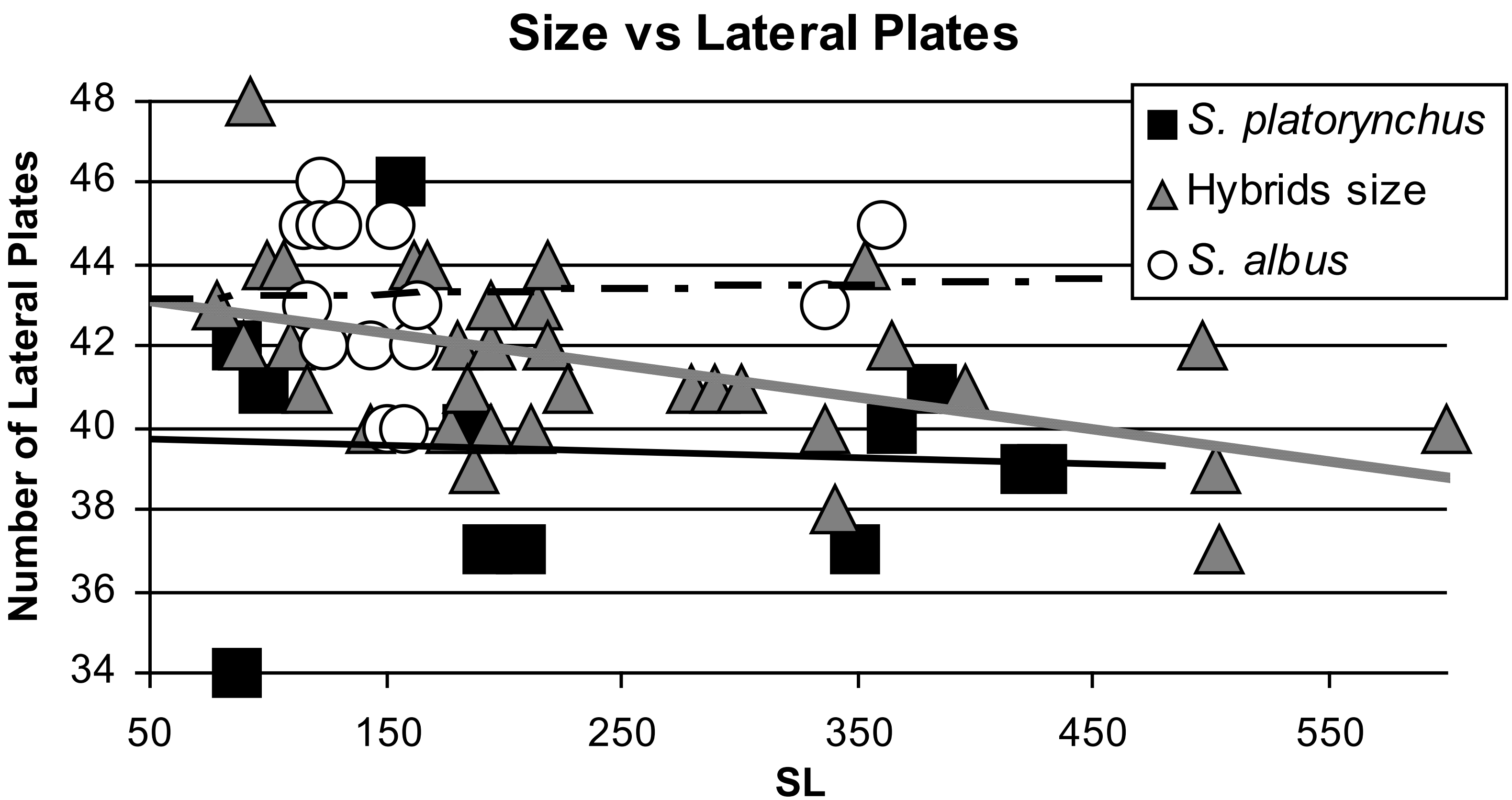

Figure 27. Relationships of lateral plates with size for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

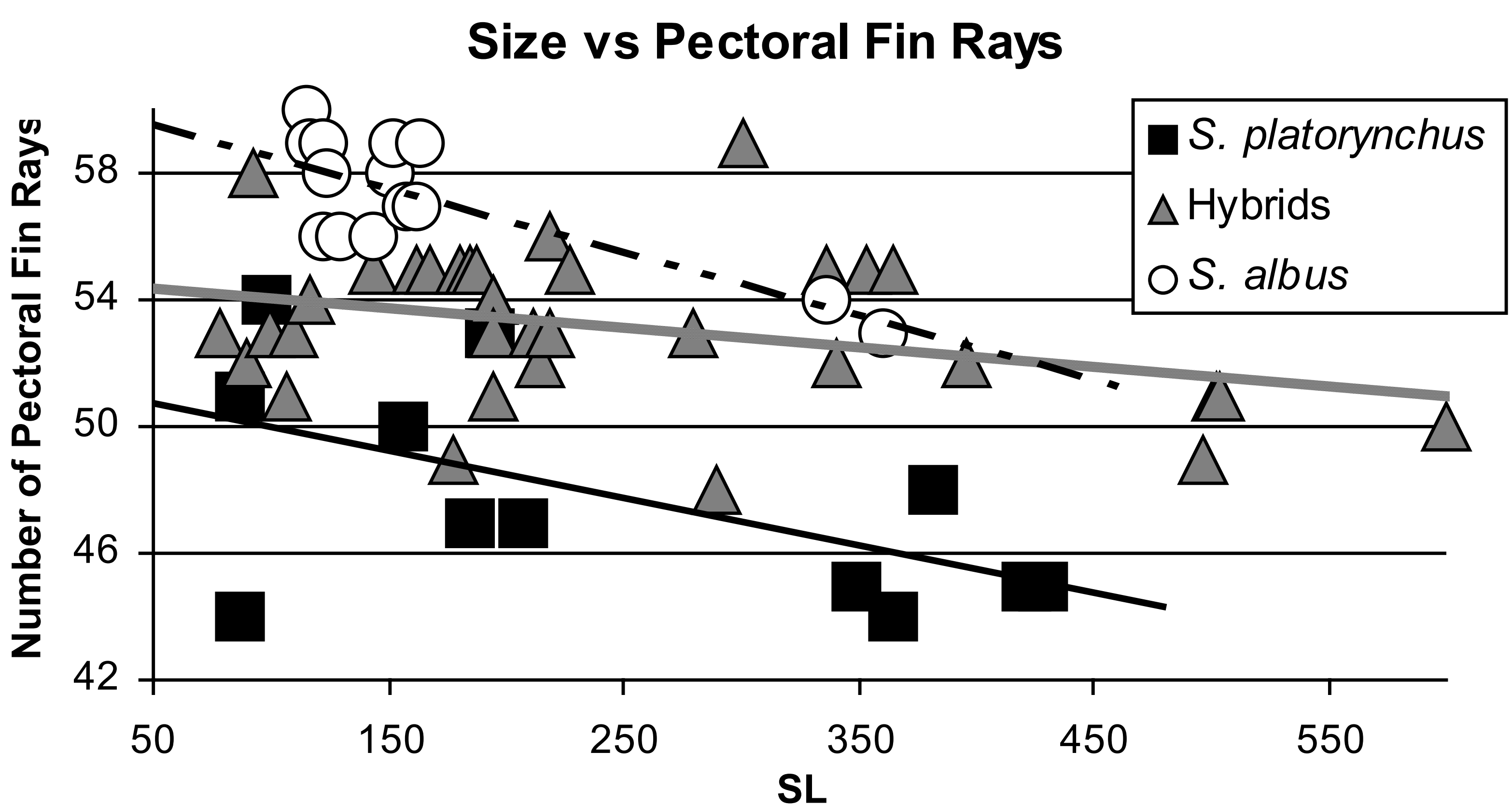

Figure 28. Relationships of pectoral-fin rays with size for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

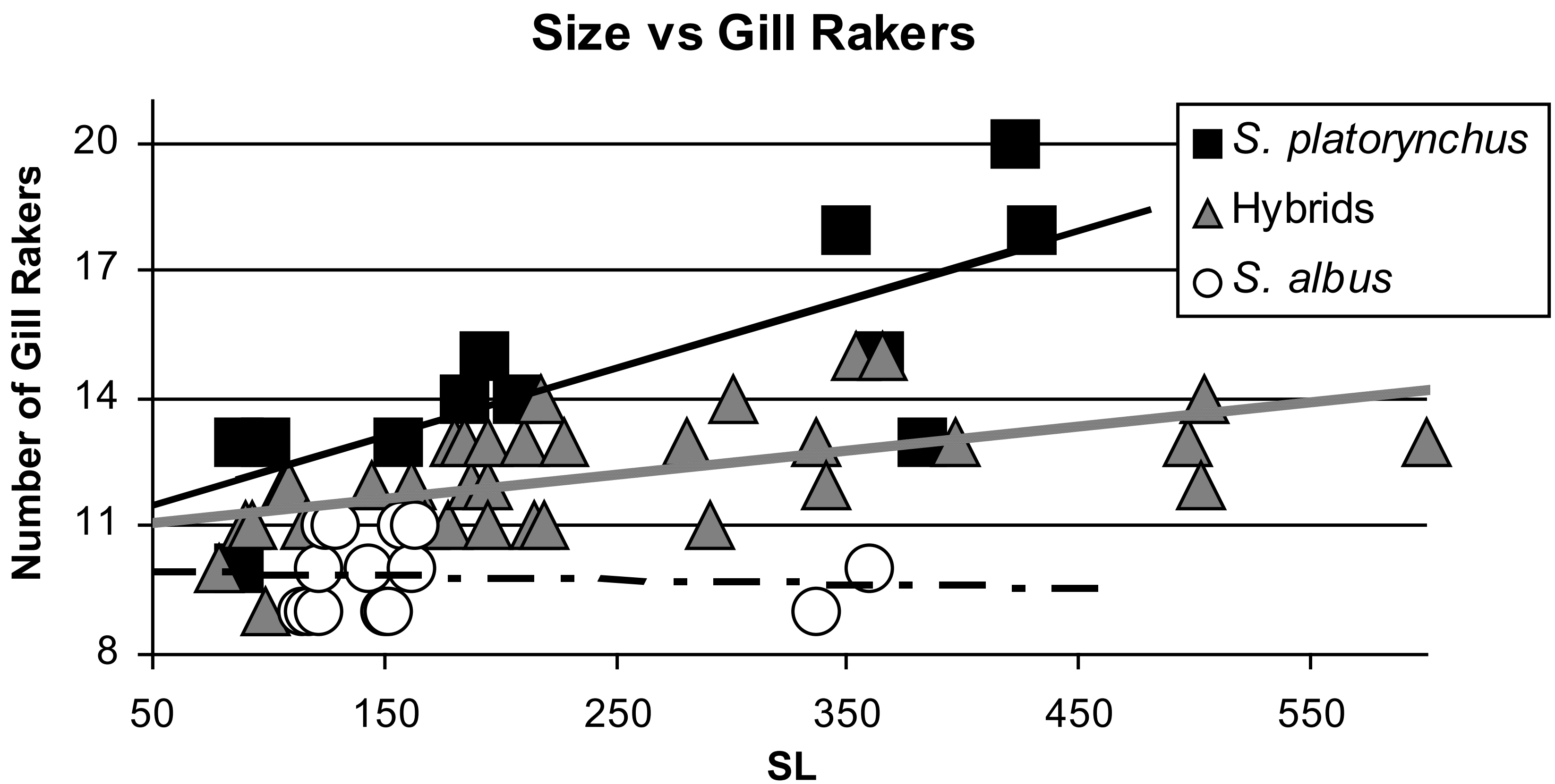

Figure 29. Relationships of gill rakers with size for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

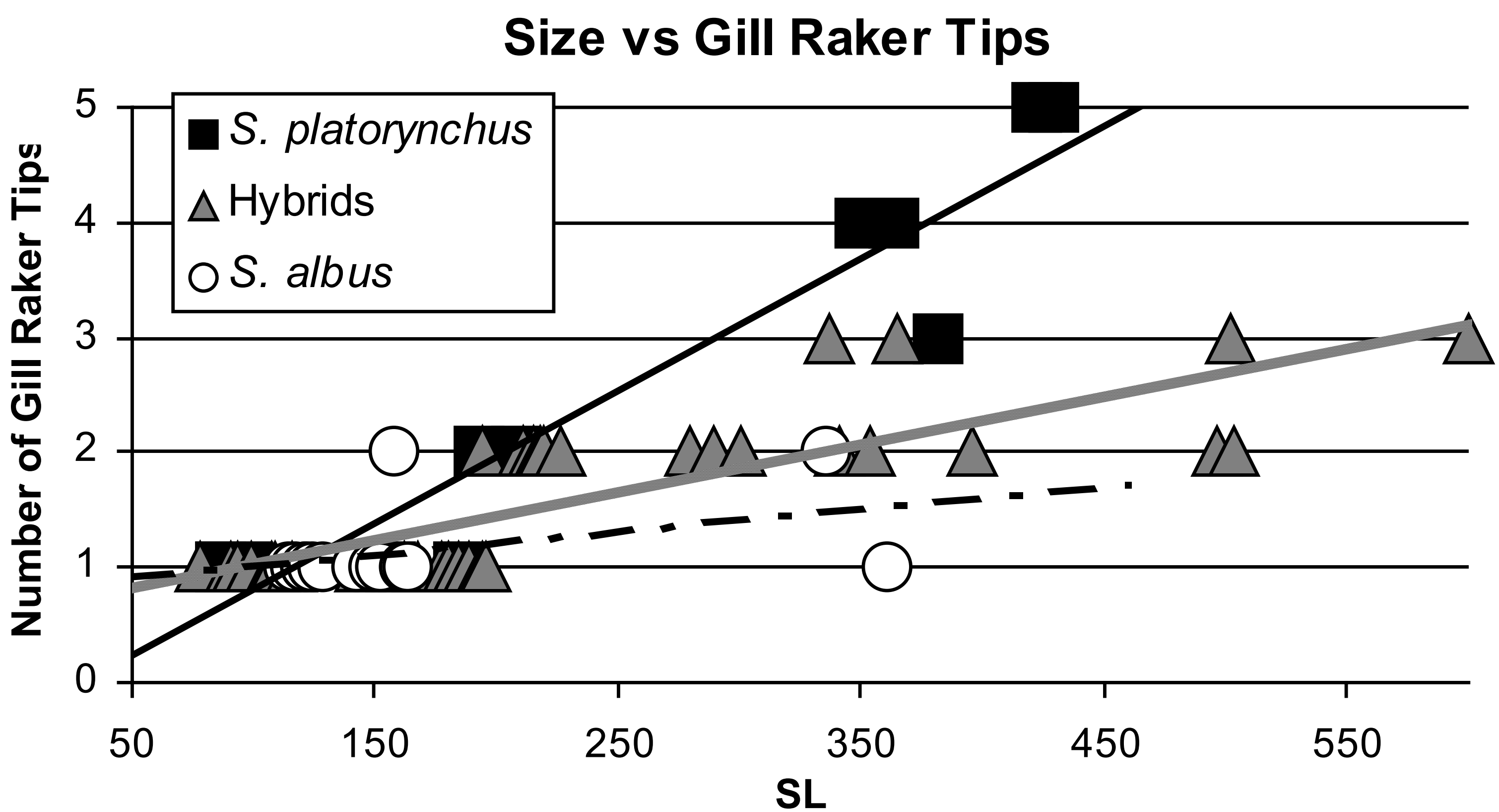

Figure 30. Relationships of gill-raker tips with size for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

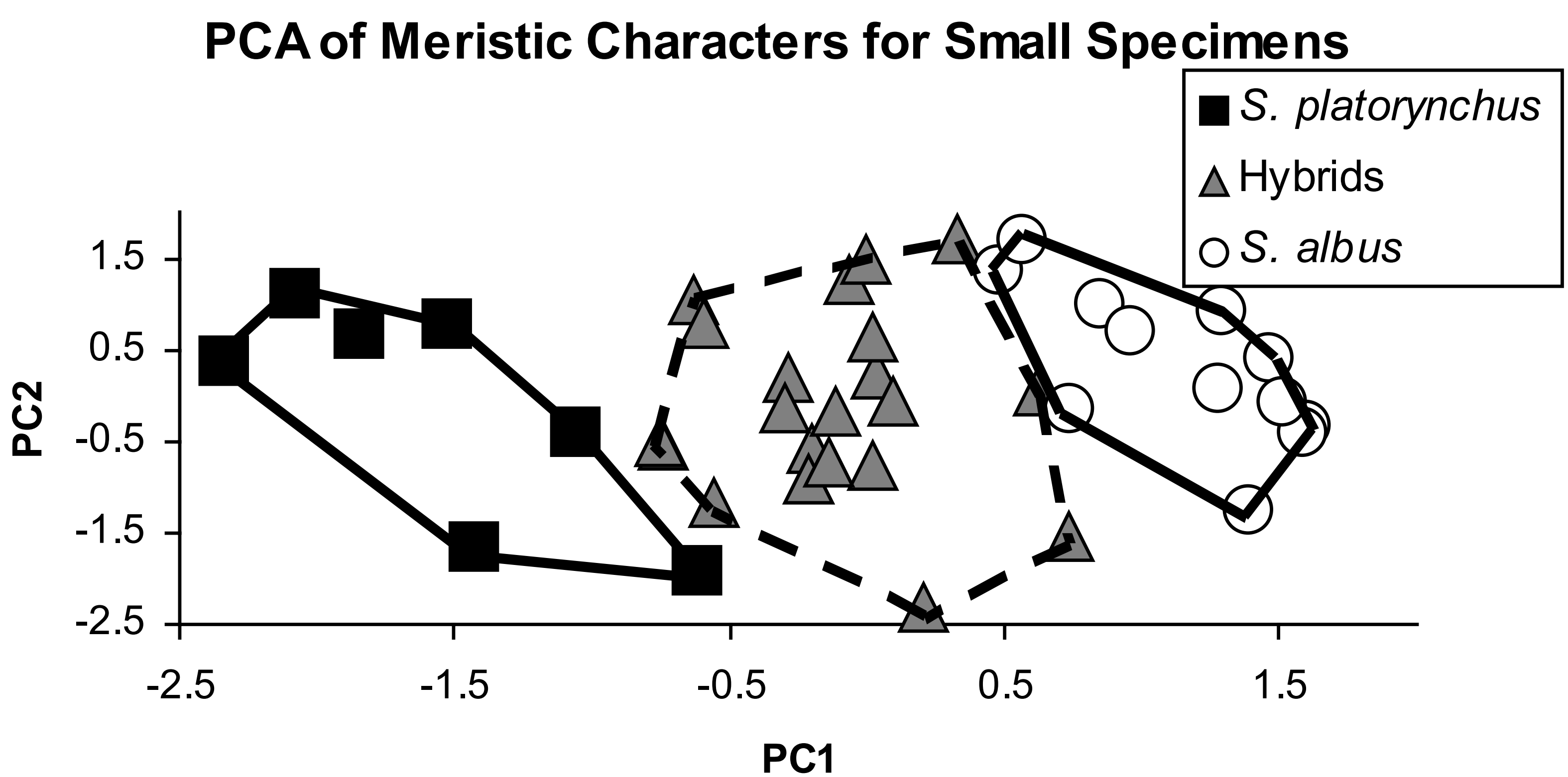

Figure 31. Principal components analysis of 13 meristic characters for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

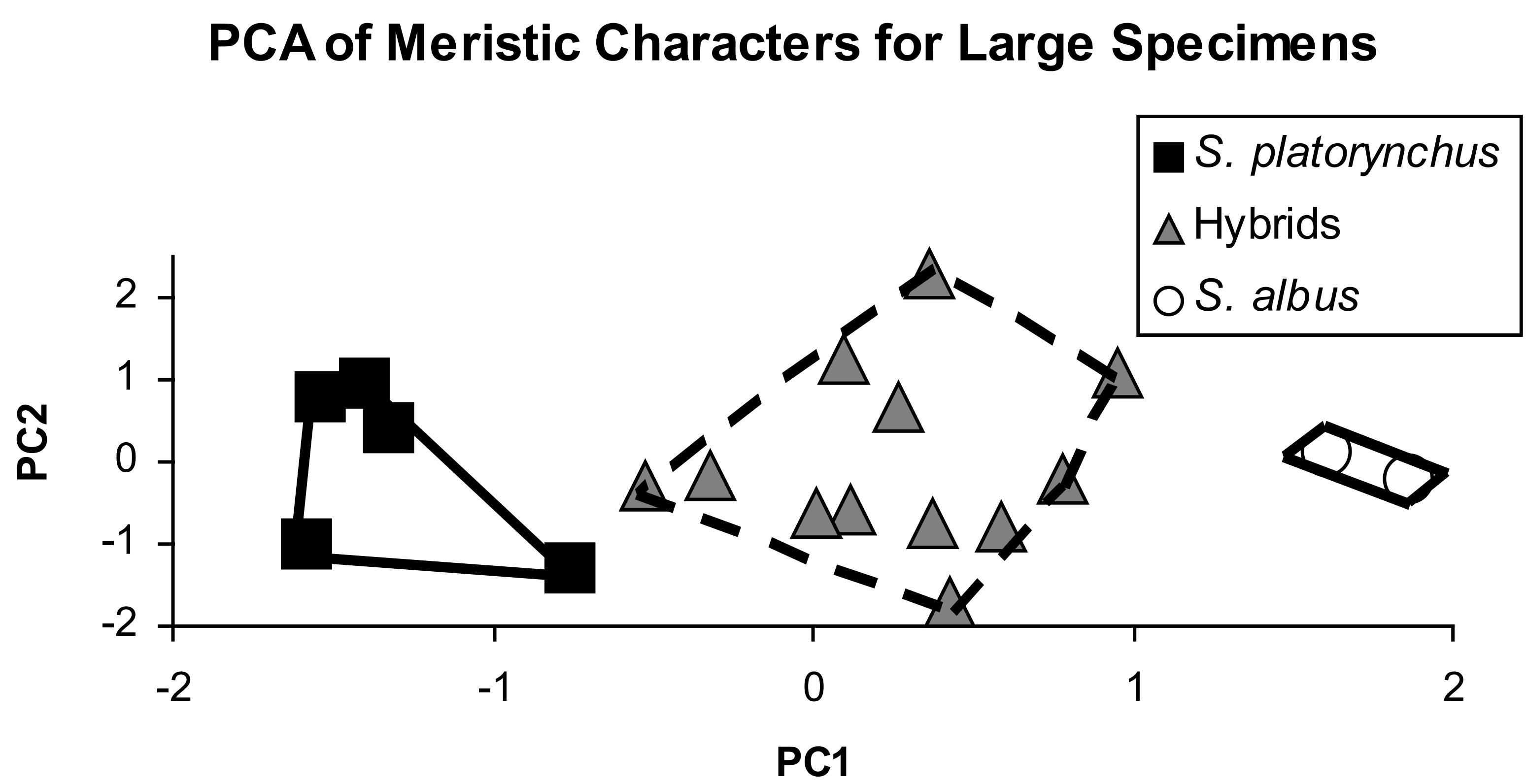

Figure 32. Principal components analysis of 13 meristic characters for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

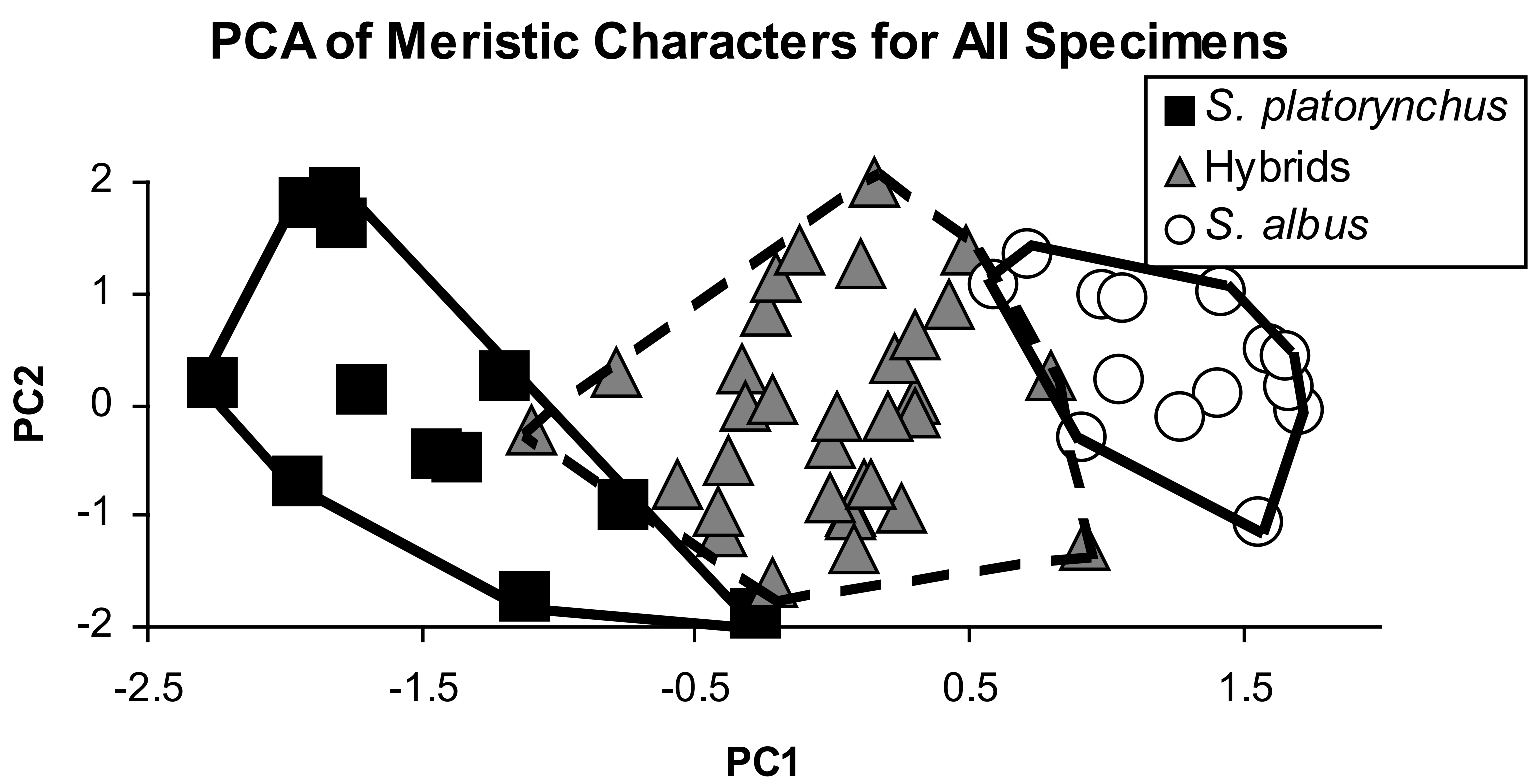

Figure 33. Principal components analysis of 13 meristic characters for all sizes of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

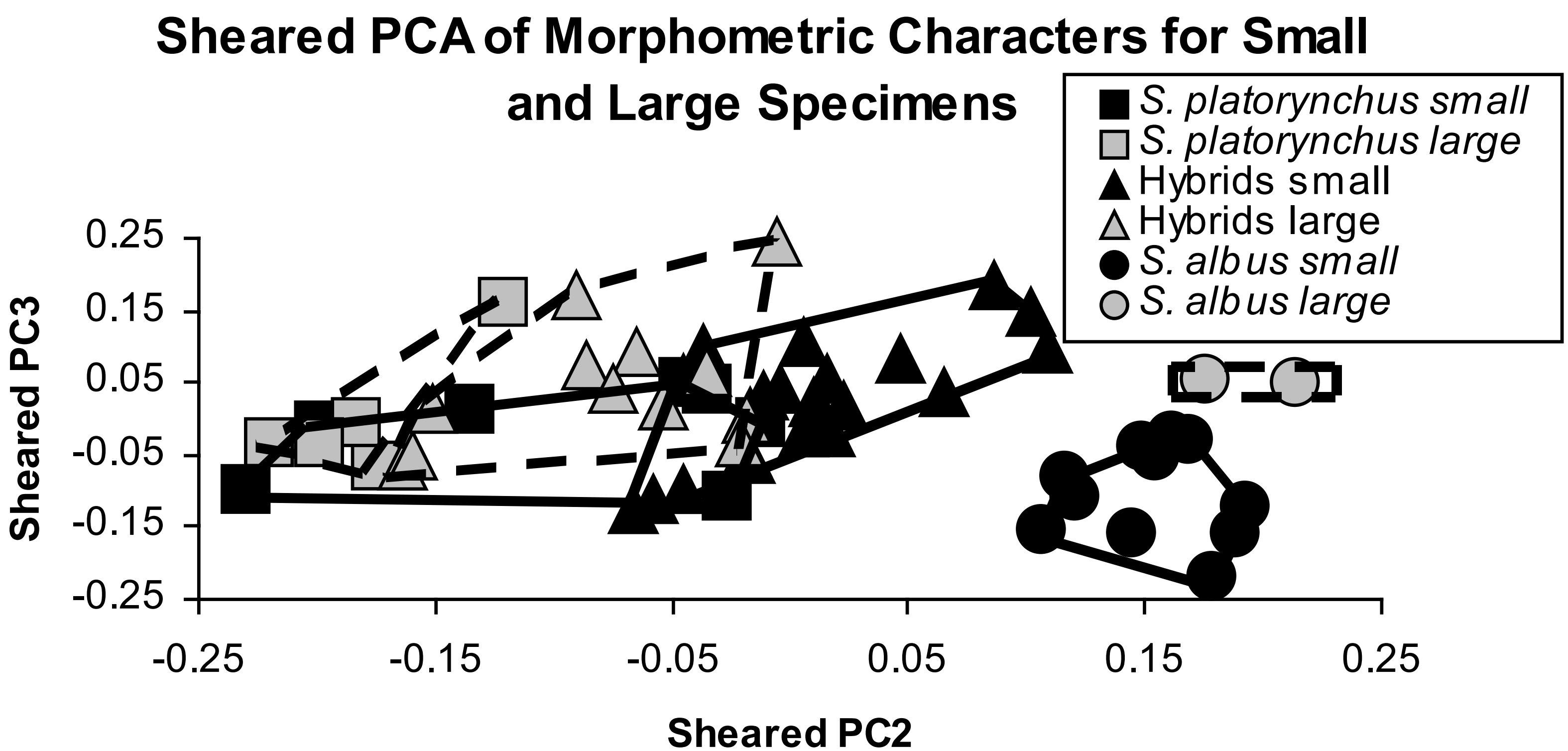

Figure 34. Sheared principal components analysis of 51 morphometric characters for small and for large size classes of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

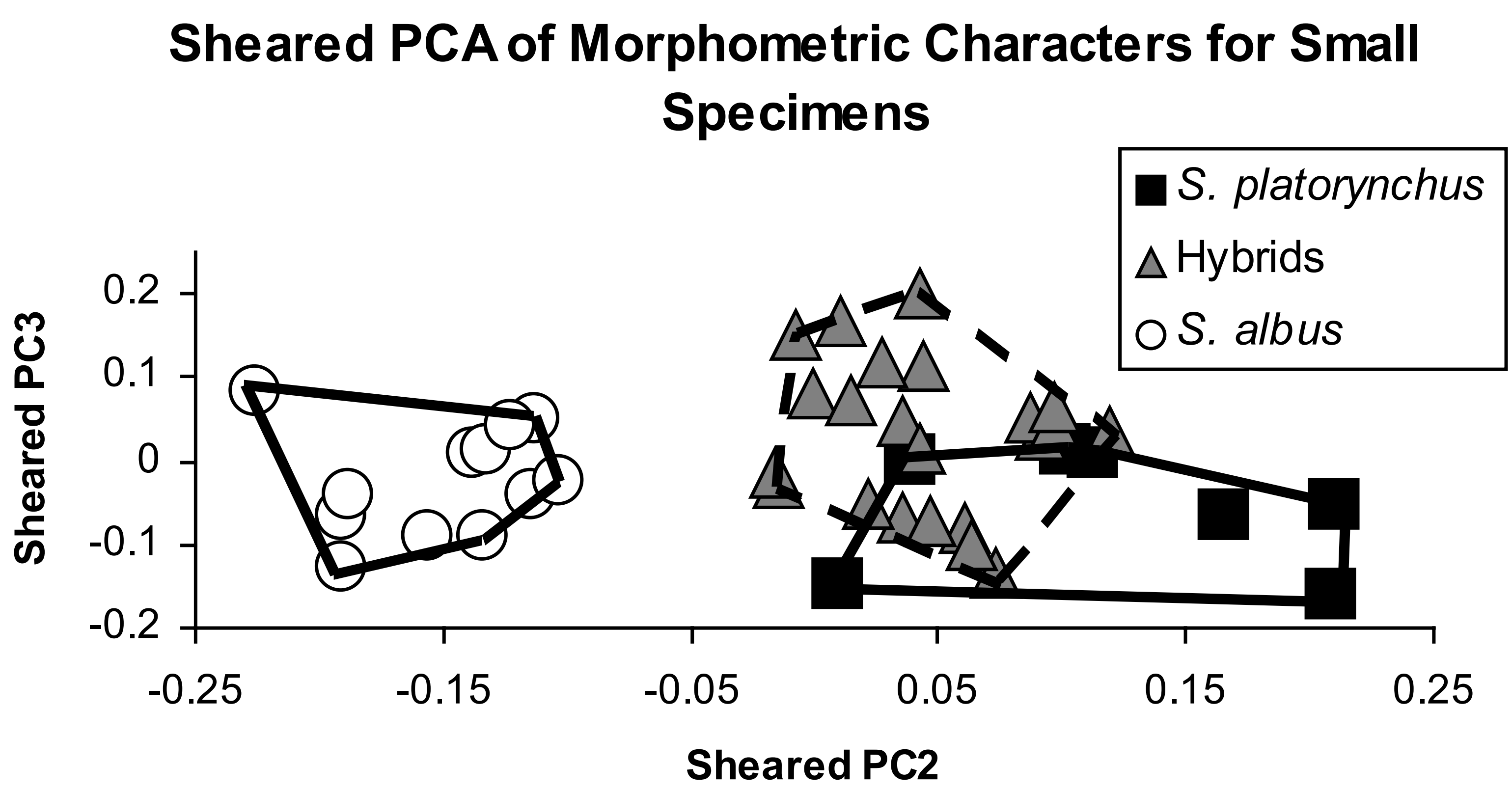

Figure 35. Sheared principal components analysis of 51 morphometric characters for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

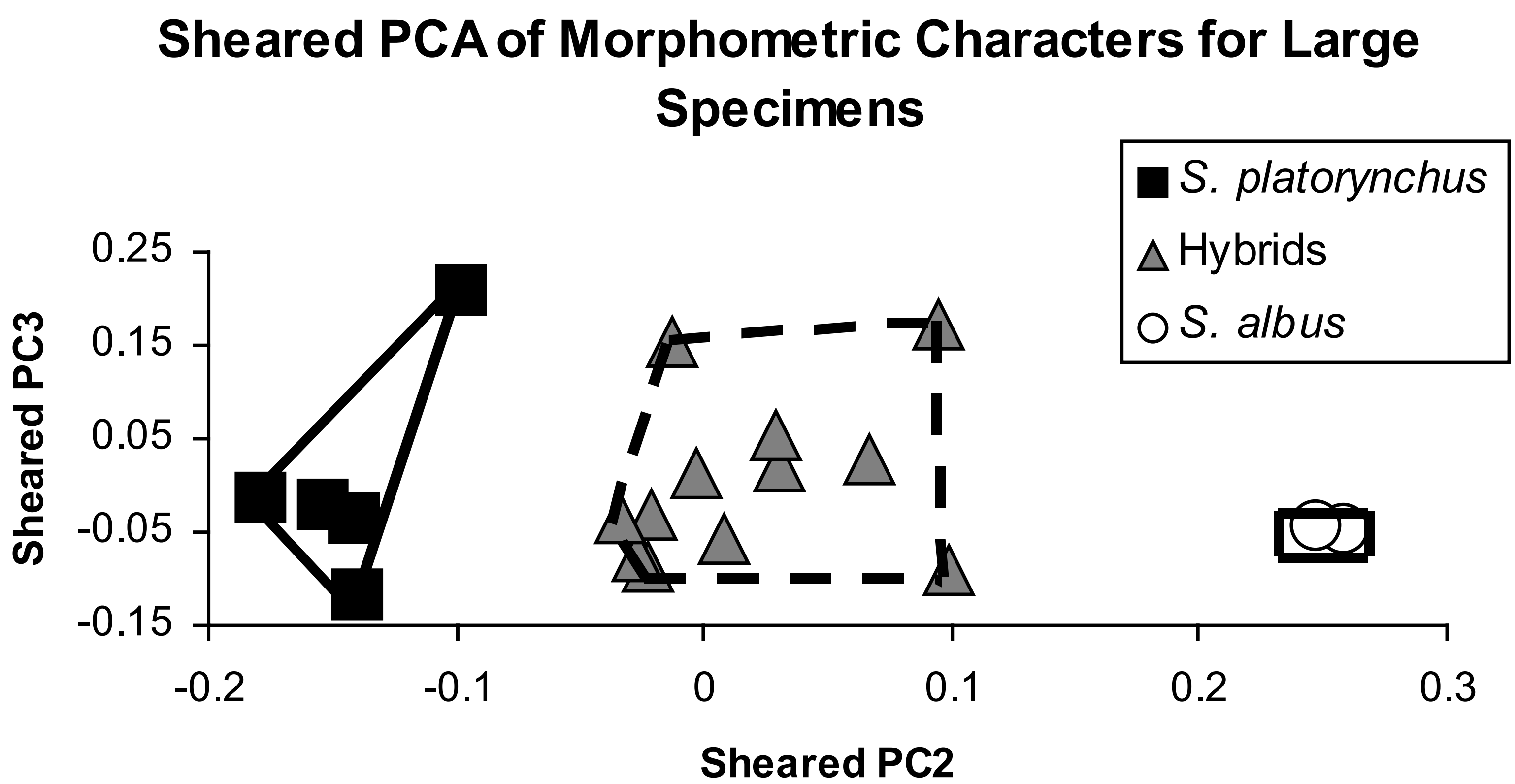

Figure 36. Sheared principal components analysis of 51 morphometric characters for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

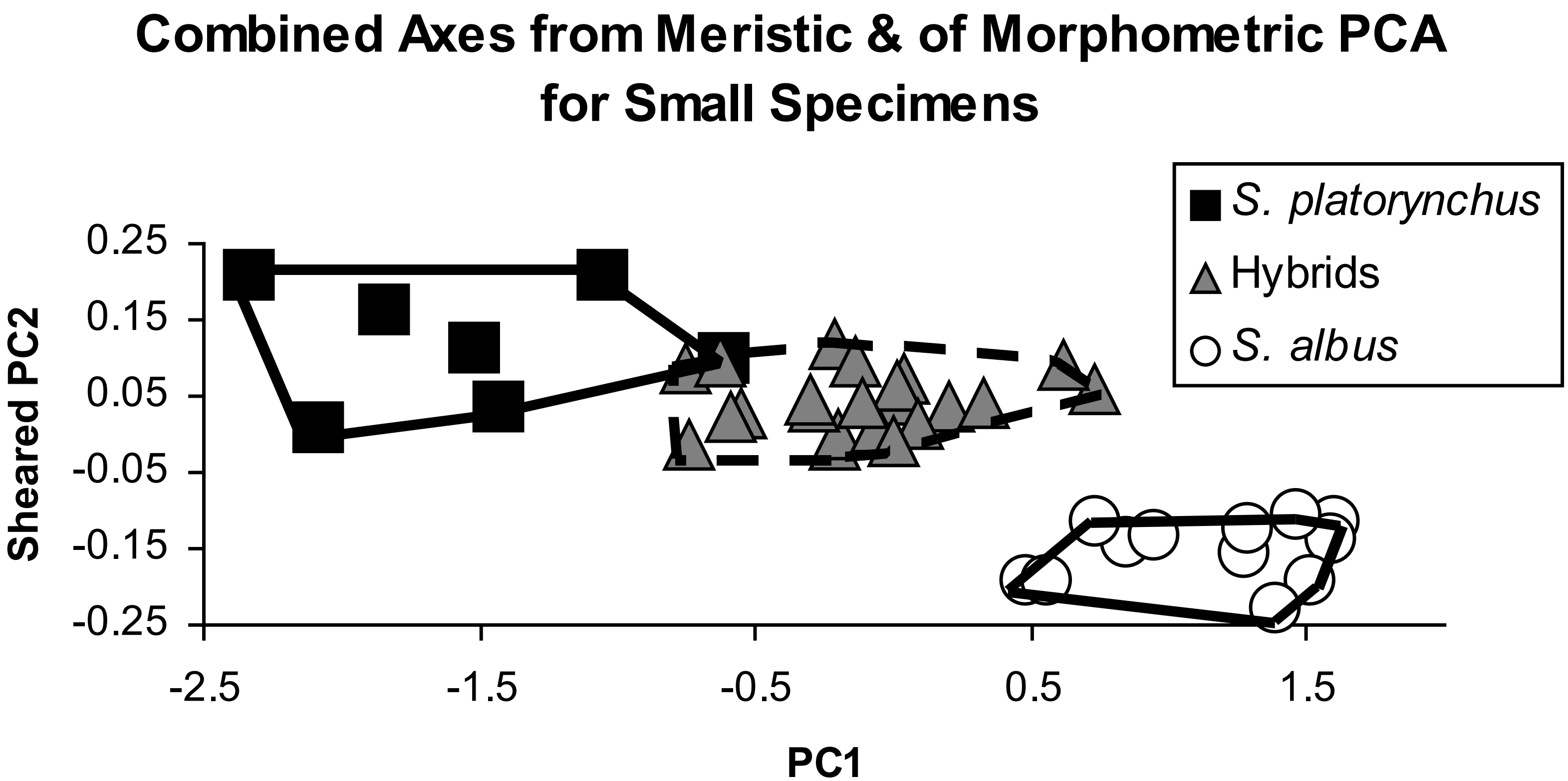

Figure 37. Axes from PC1 of principal components analysis of 13 meristic characters and PC2 of sheared principal components analysis of 51 morphometric characters for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

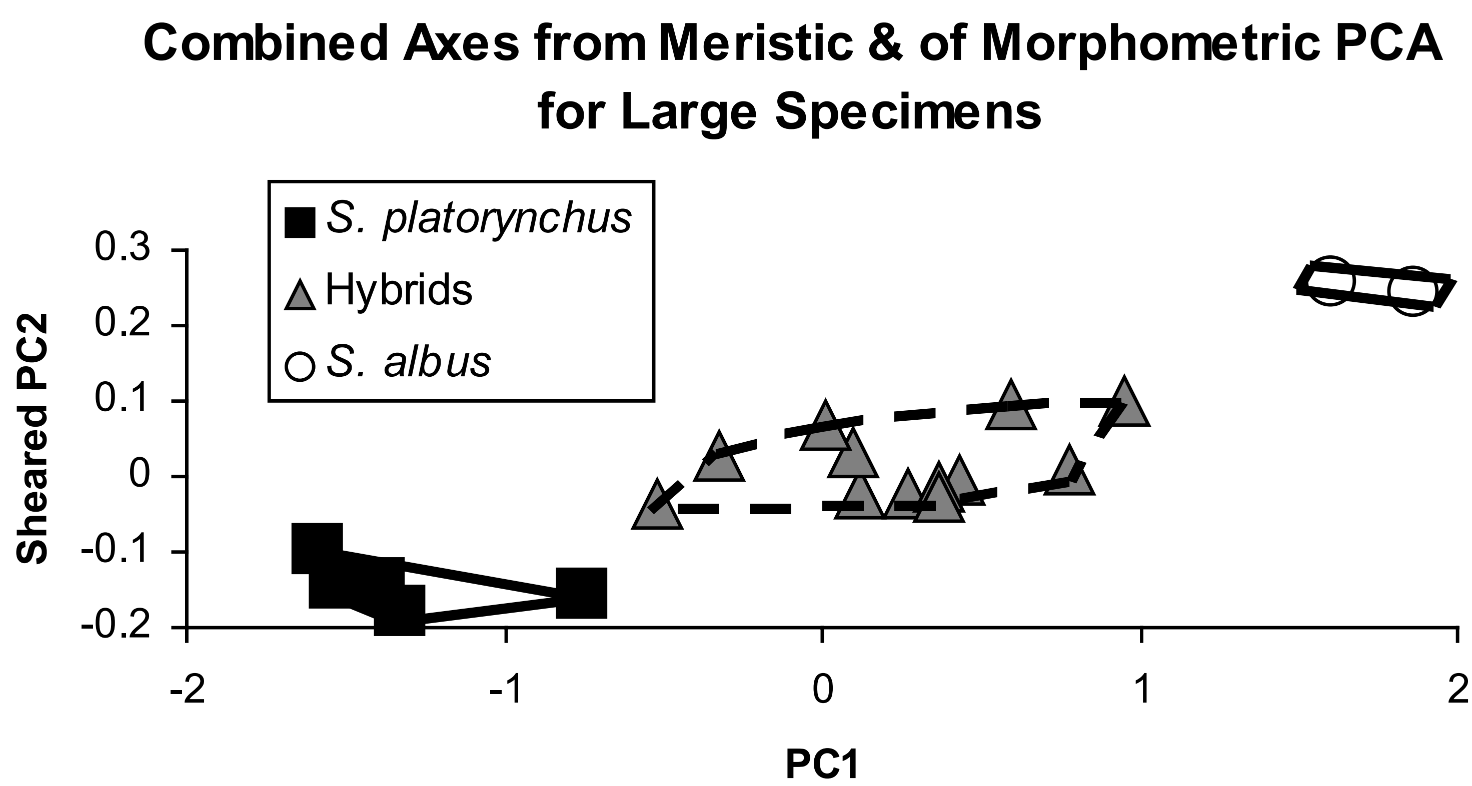

Figure 38. Axes from PC1 of principal components analysis of 13 meristic characters and PC2 of sheared principal components analysis of 51 morphometric characters for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

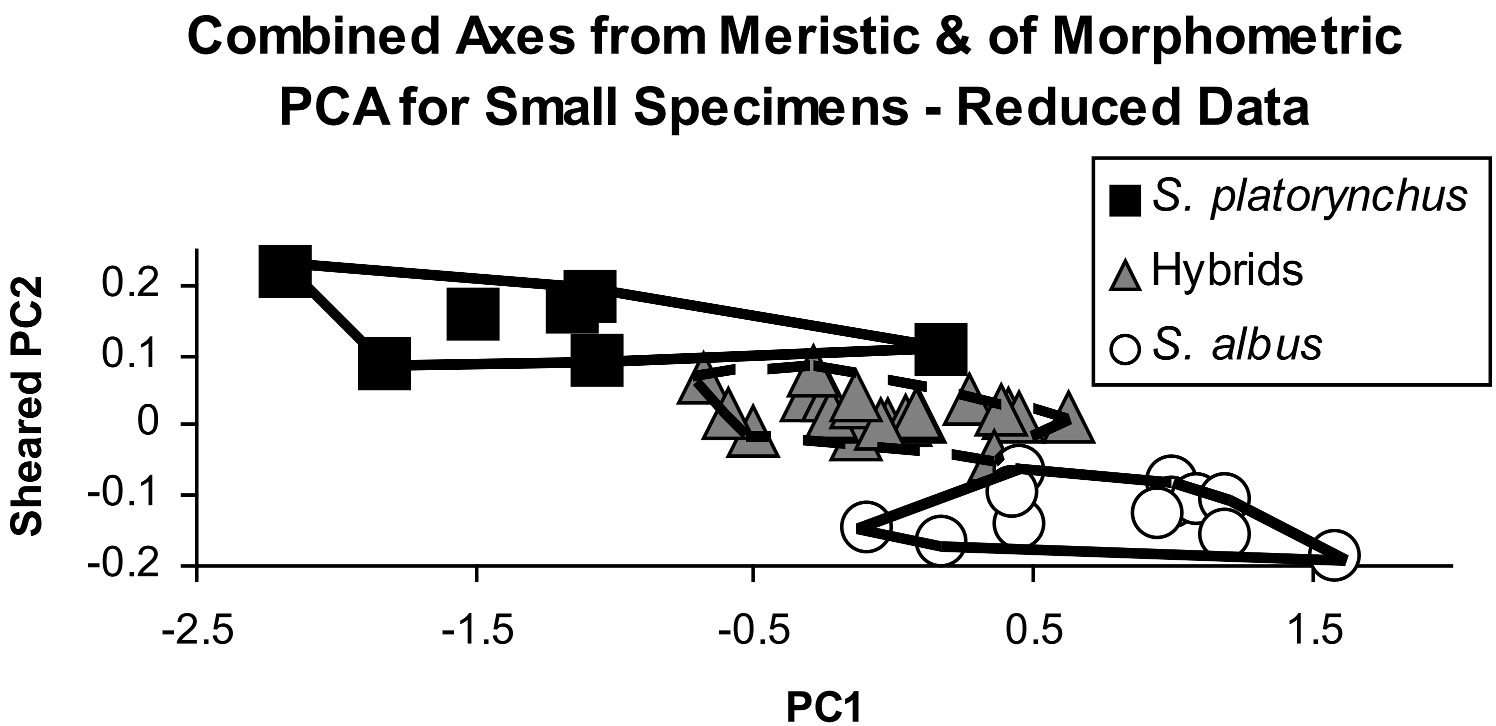

Figure 39. Axes from PC1 of principal components analysis of 6 meristic characters and PC2 of sheared principal components analysis of 12 morphometric characters for small size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

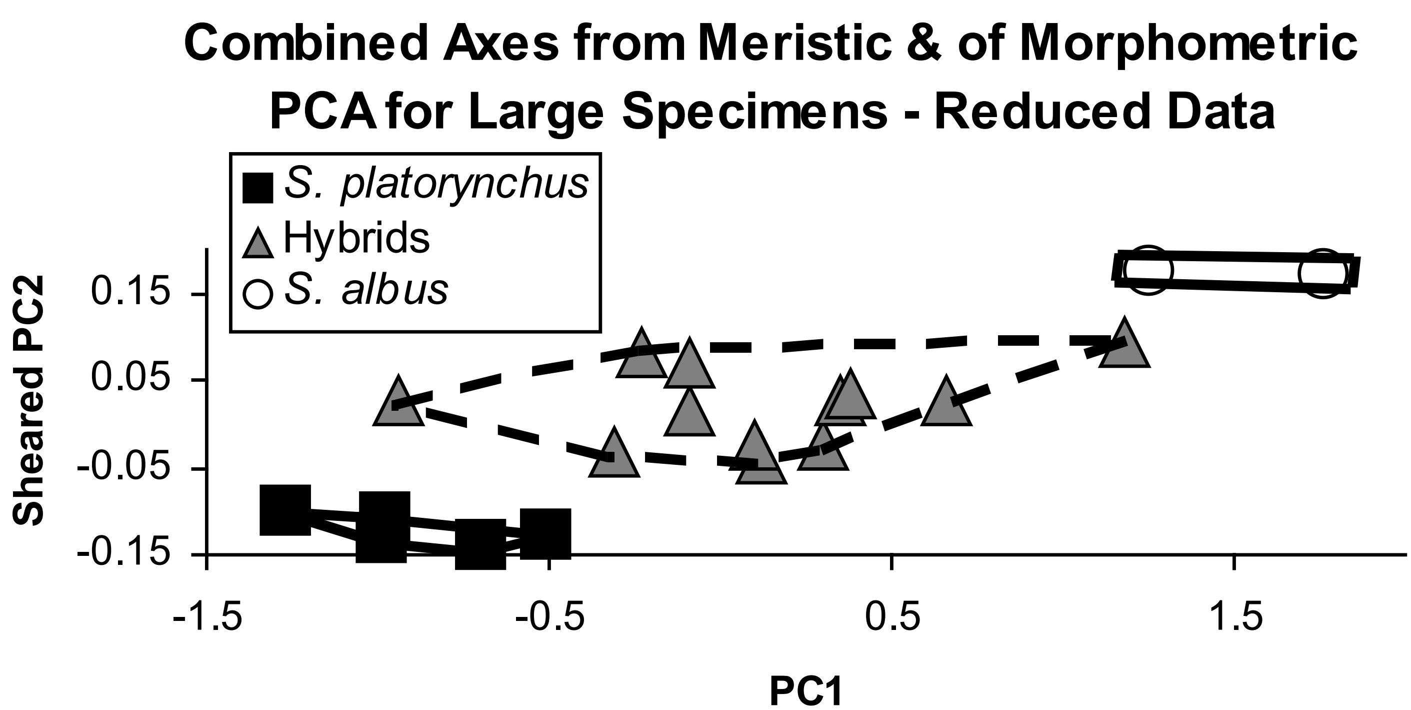

Figure 40. Axes from PC1 of principal components analysis of 6 meristic characters and PC2 of sheared principal components analysis of 12 morphometric characters for large size class of hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

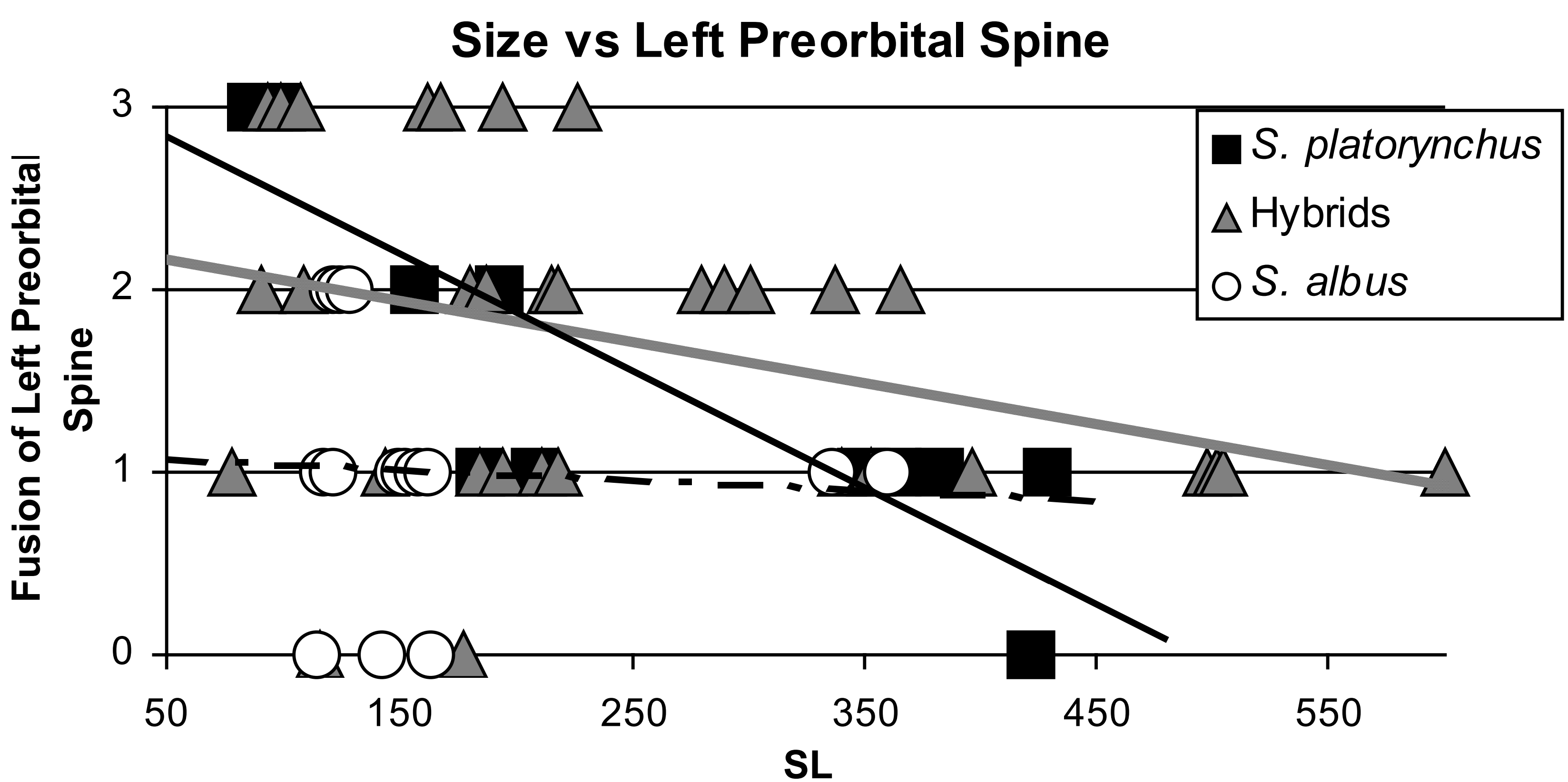

Figure 41. Relationships of left preorbital spine fusion with size for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

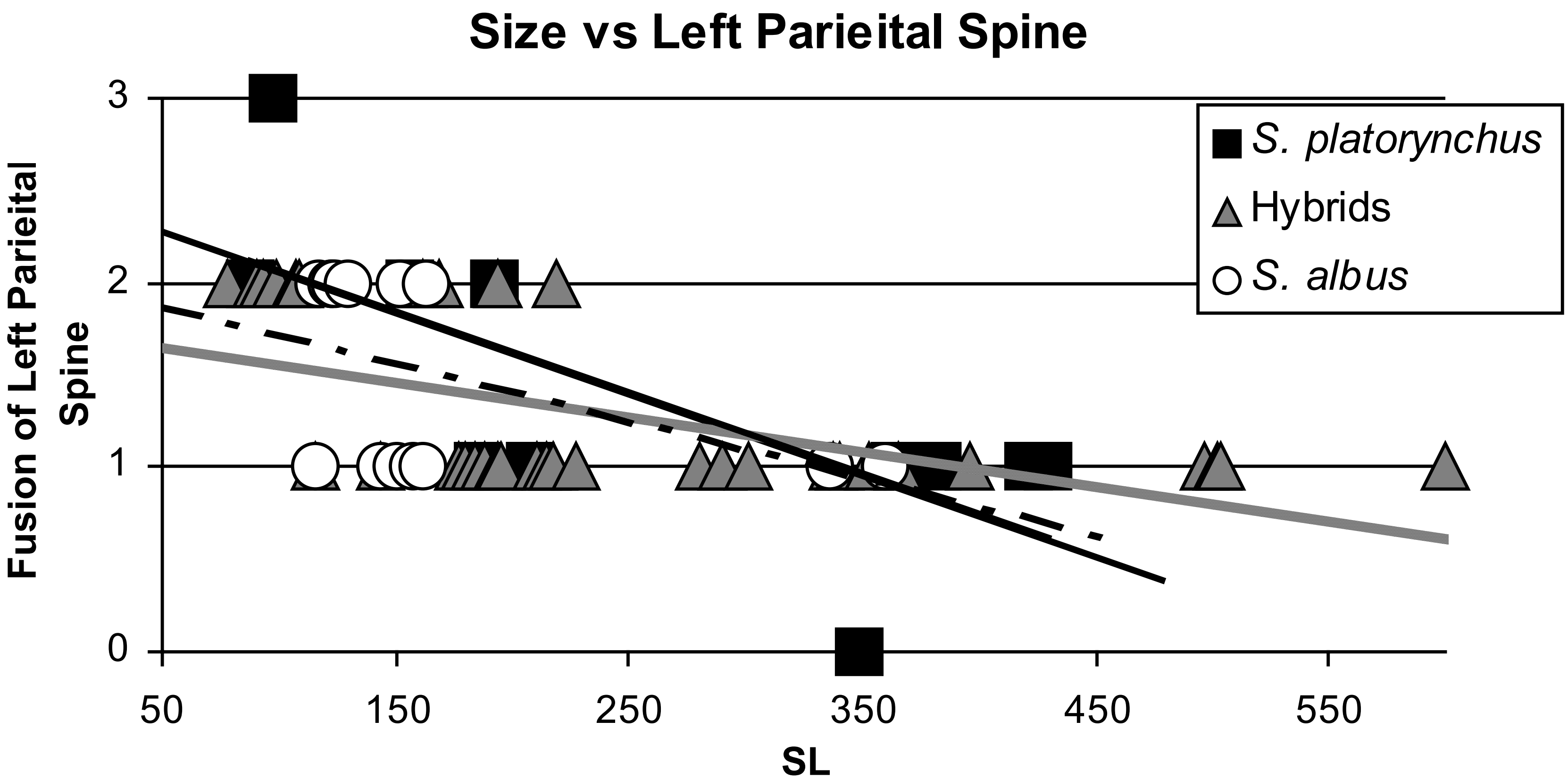

Figure 42. Relationships of left parietal spine fusion with size for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

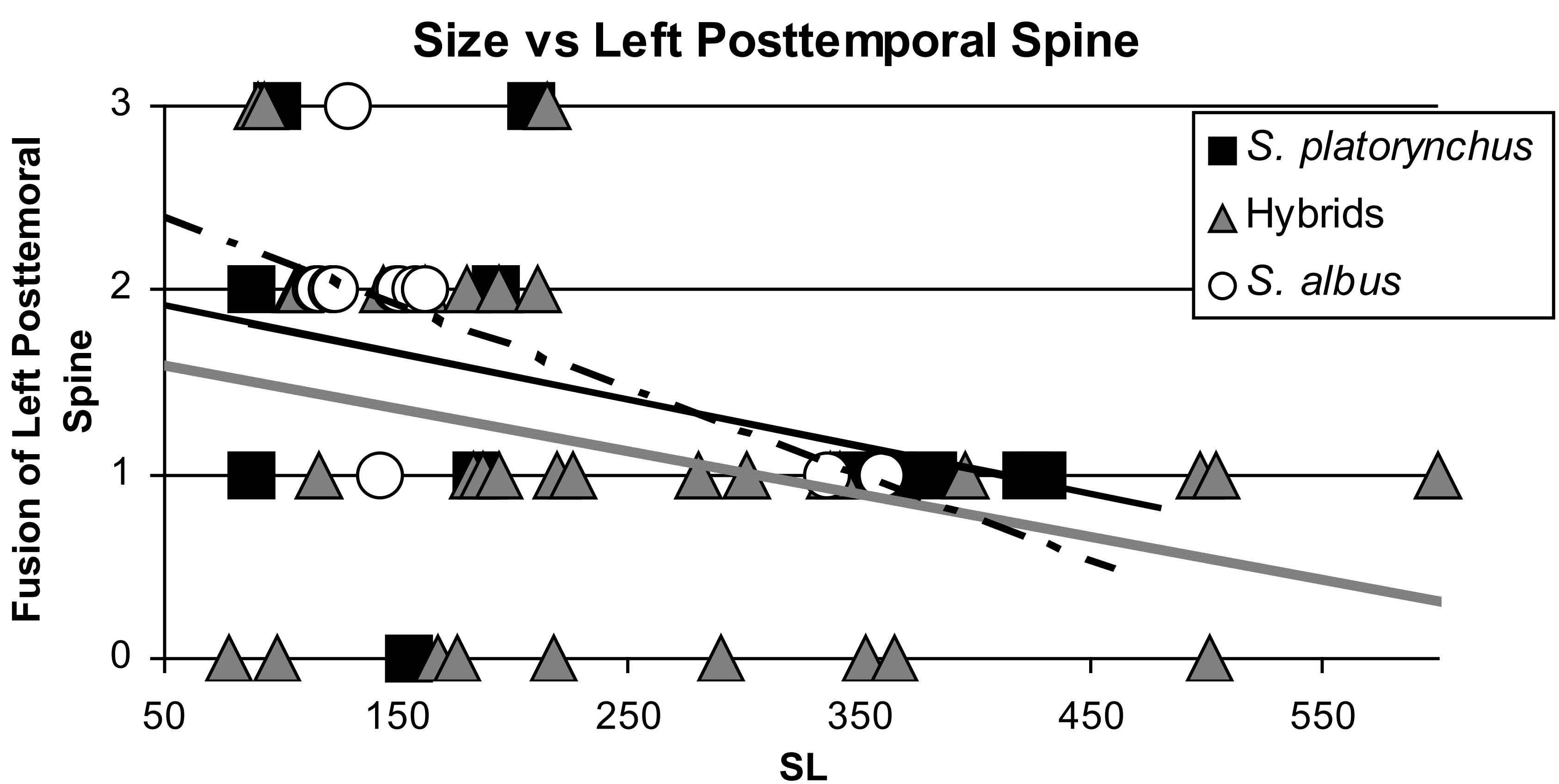

Figure 43. Relationships of left posttemporal spine fusion with size for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.

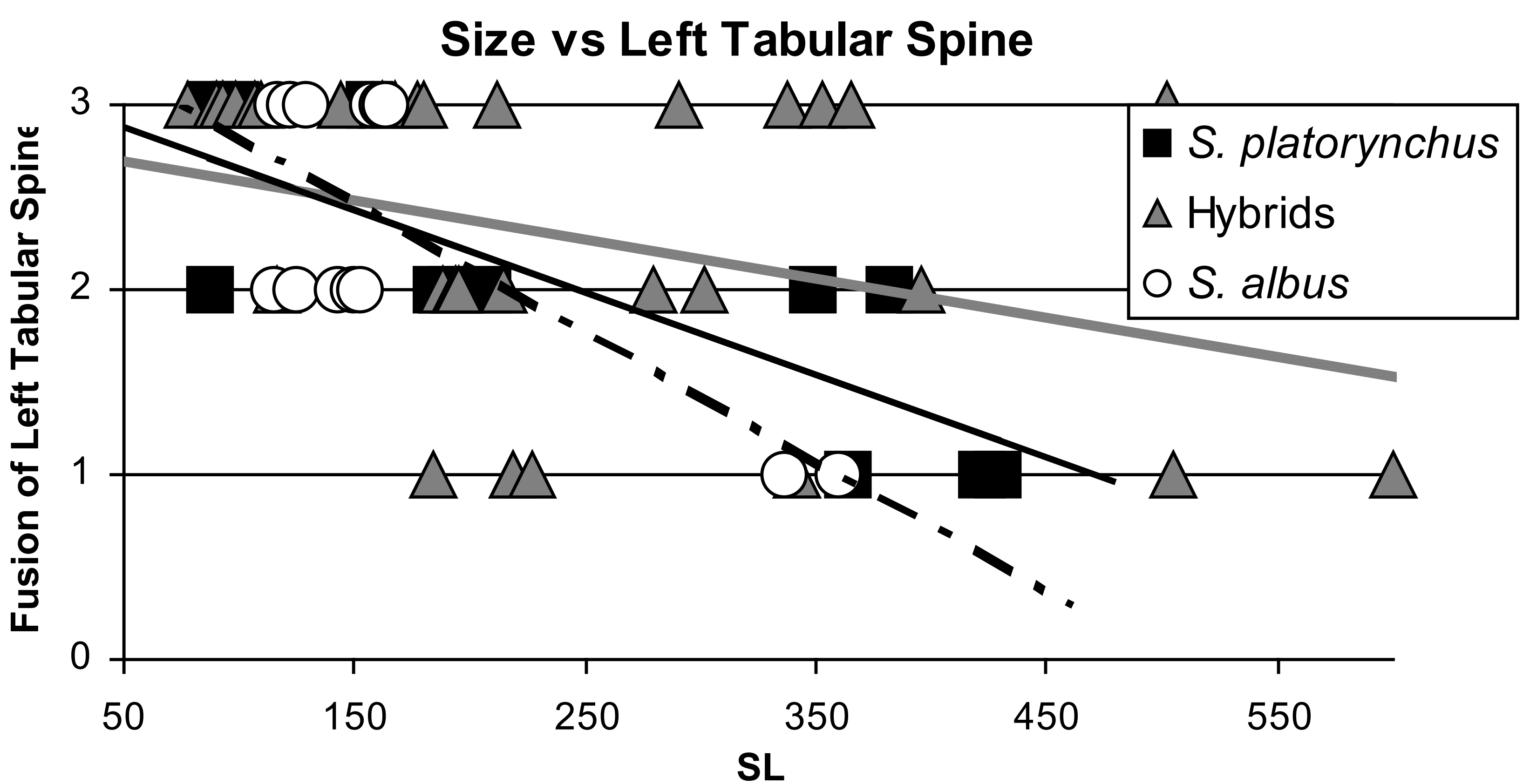

Figure 44. Relationships of left tabular spine fusion with size for hatchery-reared specimens of *Scaphirhynchus platorynchus*, *S. platorynchus* x *S. albus*, and *S. albus*.