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Multivariate Discrimination of Colorado Plateau *Gila* spp.: The "Art of Seeing Well" Revisited

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Abstract.—Fishery managers have long been troubled by phenotypic variation within and among Colorado Plateau *Gila*. The problem is twofold. From an historical perspective, there was reticence to investigate fishes long considered as "undesirable." In a taxonomic sense, there is confusion over within- and among-species variability. We document the former, then clarify the latter by applying discriminant analysis to meristic and morphometric data collected from museum specimens. We test three hypotheses: roundtail chub *G. robusta,* humpback chub *G. cypha,* and bonytail *G. elegans* are morphologically indistinguishable; juveniles are assignable to species based upon adult characters; and putative hybrids are morphologically intermediate between parental forms. Through the use of meristic characters in a nonparametric discriminant analysis, over 95% of all adults were segregated to species. By using morphometric characters, 97% could be allocated to species. *Gila robusta* was easily separated from *G. cypha* and *G. elegans.* The latter were most difficult of all species-pairs to discriminate, yet field characters still segregated them at better than 95%. A discriminant function, based upon five morphometric characters, will allocate unknowns to species. Juvenile *G. robusta* were easily discriminated (>97%), but juveniles of *G. cypha* and *G. elegans* were often misidentified as *G. robusta.* Putative hybrids were generally assigned to one or the other parental form; thus, hybrid intermediacy could not be rejected. However, paucity of hybrids weakened the test. We conclude that factors most important in segregating these species are character selection, adequate analyses, and "the art of seeing well."

The cyprinid fish genus *Gila,* endemic to western North America, exhibits a mosaic of morphological and meristic variation both within and among species. This phenotypic diversity has been attributed to numerous causes, both biological and methodological: Ecotypy–ecophenotypy (Miller 1945, 1946), hybridization (Holden and Stalnaker 1970; Dowling and DeMarais 1993), ontogeny (Douglas 1993), character selection (Douglas et al.

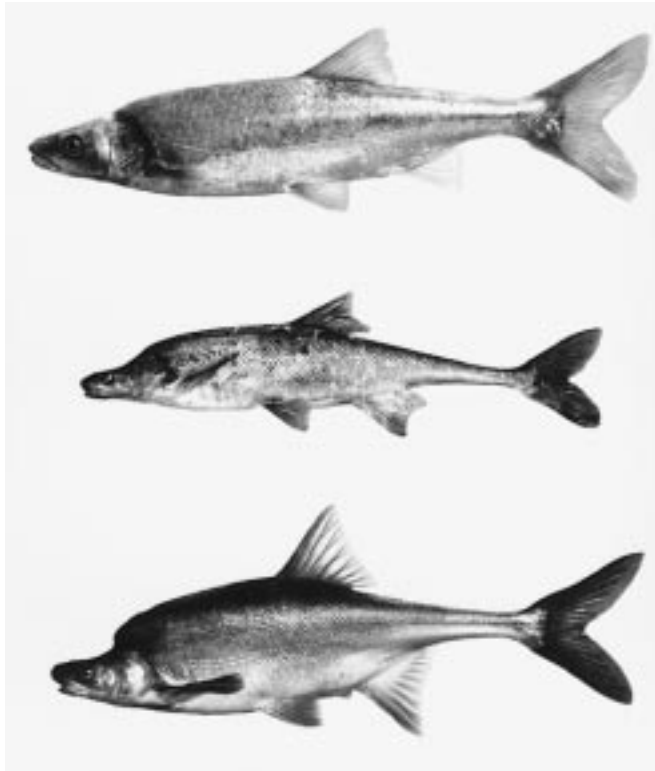* Corresponding author: m.douglas@asu.edu

FIGURE 1.—Big-river *Gila* spp. from the Colorado Plateau of southwestern North America: *G. robusta* (top); *G. elegans* (middle); *G. cypha* (lower).

1989), or weak or inadequate analyses (Douglas 1993). Particularly problematic have been ''big-river'' chubs of the Colorado Plateau (i.e., humpback chub *G. cypha,* bonytail *G. elegans,* and roundtail chub *G. robusta*; Figure 1). The ability of fishery biologists to morphologically discriminate these chubs has not markedly improved since the fish were initially described (reviewed by Douglas et al. 1989:654; Minckley 1996:18–22). If anything, it has worsened. In the last few decades, Colorado Plateau rivers have been beheaded, diverted, or dammed for human uses (Fradkin 1984), thus eliminating or compromising realized niches of these species. Forms once allopatric are now often constrained into sympatry, confounding an already complex issue by markedly increasing potentials for hybridization. Ongoing studies of these forms are further impeded because of their endangered status (Douglas et al. 1989).

Phenotypic variability in *Gila* has long been ignored by fishery managers in western North America as inconsequential to the job of managing more profitable game fishes (Holden 1991). This bias not only retarded native fish management for de-

cades but contributed greatly to the overall confusion surrounding the big-river chubs. Holden (1991) summarized (and interpreted) the philosophy of those times when he declared

In the 1950s, two forms of bonytails (a common name then generally applied to Colorado River chubs) were taxonomically recognized as subspecies—roundtail chubs (*G. r. robusta*) and bonytail (*G. r. elegans*). A third form, the humpback chub (*G. cypha*) had only recently been described (Miller 1946) and did not yet enjoy general acceptance as a valid taxon. Thus, McDonald and Dotson (1960) reported collections from the Green River in Hideout Canyon in July 1959 that included 46 ''bonytails,'' but Smith (1960) reported these same collections to include 13 humpbacks and 36 bonytails. . . . Bosley (1960) recorded all the chubs he collected as ''bonytails,'' to which he applied the name *G. r. robusta,* even though he mentioned that ''there appears to be a change in the physical characteristics of this fish in the extreme lower section of the study area,'' and presented photographs that clearly included all three species. McDonald and Dotson (1960) also acknowledged that several morphological variants existed, yet these researchers grouped all the fish into a single taxon since

they, and many other state biologists, could not see the clear-cut differences suggested by taxonomists.

Holden (1991) further noted that

this situation exemplified a fairly common phenomenon of the time. Many fishery managers either could not or would not identify the more difficult nongame fishes to species, especially closely-related minnows and suckers, or else they misidentified them. Because these species had little prominence in management decisions, they were often identified only to family even though a multitude of kinds were represented. Taxonomic concerns were the realm of taxonomists, usually housed in universities, rather than fish and game departments.

This study was undertaken to clarify morphological variability within and among big-river *Gila* of the Colorado Plateau. The initial hypothesis under test is that the study species are morphologically indistinguishable. This aspect (i.e., the swamping of between-species by within-species variance) baffled fishery biologists for years and led to the management philosophy described above. Three different data sets were employed in this study (i.e., continuous morphometric measurements, discrete meristic characters, and an amalgam of the two suitable for field use). Results were compared and contrasted to determine the efficacy of each character set in discriminating species. In addition, a discriminant function generated for all three species from field data was used to classify juveniles (<180 mm standard length, SL) and putative hybrids. Two hypotheses are tested here. The first argues that characters which discriminate adults will also discriminate juveniles. This issue, an addendum to the original problem of within-species variability, also plagued early researchers (Vanicek and Kramer 1969:195). The second hypothesis is that putative hybrids are intermediate in their phenotypes (as per Hubbs et al. 1943) and cannot be unambiguously assigned to one or another of the parental species.

Methods

Specimens, measurements, and data sets.—Museum specimens of the three *Gila* species (to 1978) were used in this study. These included the following adults and juveniles: *G. robusta* (81 adults, 106 juvniles), *G. cypha* (58 adults, 30 juveniles), and *G. elegans* (28 adults, 6 juveniles). An additional 17 ''hybrids,'' were examined, as were three *G. seminuda* (a hybrid species of *G. robusta* × *G. elegans* ancestry; DeMarais et al. 1992). A series of 25 morphometric and 10 meristic variables were collected from each specimen (Appendix 1). A list-

ing of specimens examined is provided in Appendix 2.

Very few values were missing from the original data (for *G. robusta,* 50 out of 2,835 metrics [1.76%]; for *G. cypha,* 20 out of 2,030 [0.98%]; and 18 out of 980 [1.83%] for *G. elegans*). Virtually all missing data were morphometric; only four individuals lacked either the left pharyngeal bone or its accompanying teeth. Missing values were estimated from other specimens of the same species by linear regression of the character under consideration onto the character that explained the greatest proportion of its variance (G. D. Schnell, University of Oklahoma, Missing Data Estimator program, unpublished). This approach has been used successfully in other morphometric studies in which missing values were encountered (see Douglas et al. 1984, 1992; Schnell et al. 1985).

Morphometric characters were then size-adjusted by subtracting $\log_{10}$ of each measure from $\log_{10}$ of the same individual's standard length. Ratio adjustment is supported for two reasons. First, the null hypothesis of collinearity and zero-intercept could not be rejected in any of the comparisons between ratio elements, based upon Bonferroni-adjusted criteria. When such null criteria are met, ratios correctly remove effects of overall size (Jackson and Somers 1991). However, the most important reason for using this technique is that, when coupled with a discriminant function (below), it provides field workers with a simple method for classifying *Gila* specimens in the field, one that requires only a caliper and a calculator. The complicated, computer-driven methods of multivariate size correction do not allow this (noted also by McElroy et al. 1997).

The total data were partitioned into three subsets. The 10 meristic characters formed one subset, and the 25 morphometric characters were a second. The third subset was composed only of those variables that could be measured in the field. As such, it contained 3 (of 10) meristic characters and 19 (of 25) morphometric characters (Appendix 1).

Discriminant analyses and functions.—For a linear discriminant analysis to be optimal (i.e., to provide a classification that minimizes probability of misclassifications), certain data assumptions must be met. Each group must be a sample from a multivariate normal population; population covariance matrices must all be equal; and group sample sizes must be similar. To avoid these difficulties, a nonparametric discriminant analysis (PROC DISCRIM; SAS 1989) was used to evaluate specimens for their inclusiveness in desig-

nated groups. This analysis was run for all species-pairs and for the three species together with each data type (meristic, morphometric, and field). A jackknife procedure was used in each analysis to estimate error probabilities for associating individuals to species.

The nonparametric analysis cannot produce a discriminant function with which to classify unknowns. To correct this, and thus to classify juveniles and unidentified specimens (as per Sofield et al. 1984), a parametric discriminant analysis (PROC DISCRIM; SAS 1989) was performed with only five morphometric field characters (see below) that maximally discriminated among the three species and a variance–covariance matrix pooled among species. Again, a jackknife procedure estimated error probabilities for the resulting discriminant function. Kappa, a chance-corrected statistic to compare actual and predicted group membership (Titus et al. 1984; applied in Douglas 1993) was also calculated. A canonical variate analysis (again, with the five field characters above) was then performed to test significance of the first three canonical variates and to plot them in three-dimensional space.

To test efficacy of the five-character field subset to correctly segregate individuals, the parametric discriminant analysis was run a second time, but with equal sample sizes among species (i.e., $N = 28$ for each) and within-group (rather than pooled) variance–covariance matrices. To make the test rigorous, those 28 *G. robusta* and *G. cypha* closest to the center of the combined distribution were selected (i.e., those individuals putatively most difficult to discriminate). All *G. elegans* were used.

Results

Data Normality and Transformation

Only one meristic count (of 10) was normally distributed in each species: gill rakers in *G. elegans* and lateral-line scales in *G. robusta* and *G. cypha.* Various transformations were unsuccessful in normalizing the other nine meristic counts. The number of nonnormal morphometric characters was 5 for *G. robusta*, 4 for *G. cypha*, and 10 for *G. elegans*. Width of left pharyngeal bone and depth of skull's frontal depression were nonnormal in all three species. Bony interorbital width, tip of snout to occiput, and length of upper jaw were nonnormally distributed in two of the three species.

Transformed morphometric data were also tested by species for deviations from normality. For *G. robusta,* three of the original five nonnormal characters remained so after transformation, while two (of four) and zero (of 10) remained so for *G. cypha* and *G. elegans,* respectively. Only one normally distributed character (pectoral fin length) became nonnormal in two of three species following transformation. Homogeneity of within-species morphometric covariance matrices was also tested for and subsequently rejected. Thus, a pooled variance–covariance matrix was used to derive a discriminant function for species separation.

Discrimination between Study Species

All 109 specimens of *G. robusta* and *G. elegans* were discriminated 100% of the time by either two meristic characters (dorsal rays and gill rakers) or a single morphometric character (depth of caudal peduncle; Table 1). With field data, depth of caudal peduncle again proved maximally discriminatory.

In all, 98.8% (85 of 86) of *Gila cypha* and *G. elegans* were discriminated by three meristic characters (Table 1). This included 100% (28 of 28) of *G. elegans* and 98.3% (57 of 58) *G. cypha.* With the use of only morphometric characters, 96.5% (83 of 86) of all individuals were correctly allocated. *Gila cypha* was correctly designated in 98.3% of the cases (57 of 58), and *G. elegans* in 92.9% of cases (26 of 28). Field characters clearly separated the two species in 95.3% of cases (82 of 86), with 98.3% of *G. cypha* allocated (57 of 58) and 89.3% of *G. elegans* (25 of 28). Only one individual (of eight) allocated to the ''wrong'' species was shared among the three data subsets.

Three meristic counts separated *G. robusta* and *G. cypha* in 95.7% of cases (133 of 139; Table 1). *Gila cypha* was correctly designated in 100% of cases (58 of 58), and *G. robusta* in 92.3% (75 of 81). With only morphometric variables used, 99.3% (138 of 139) of individuals were correctly designated; this amounted to 100% (81 of 81) of *G. robusta* and 98.3% (57 of 58) of *G. cypha.* When the field subset was used, 99.3% of all specimens were again correctly designated to species (98.8% [80 of 81] of *G. robusta* and 100% [58 of 58] of *G. cypha*). None of eight specimens incorrectly allocated among species was shared between the three data subsets.

When the three species were evaluated together, five meristic characters correctly designated 92.2% (154 of 167) of all individuals. *Gila robusta* was assigned in 91.4% of cases (74 of 81), *G. cypha* in 89.7% (52 of 58), and *G. elegans* in 100% (28 of 28). When only morphometric characters were used, specimens were designated in 95.6% of cases (131 of 137). *Gila robusta* was allocated in 100%

TABLE 1.—Results from discriminant analysis based on meristic (10 variables), morphometric (23 $\log_{10}$-transformed variables), and field-usable (23 meristic and morphometric variables) characters that were used to discriminate among *Gila robusta, G. elegans,* and *G. cypha.* Percent discrimination is presented for each model within each contrast.

Species compared	Characters		
	Meristic	Morphometric	Field-usable
G. robusta/*G. elegans*	Dorsal rays Gill rakers (2nd arch)	Caudal peduncle depth	Caudal peduncle depth
Discrimination	(100%)	(100%)	(100%)
G. cypha/*G. elegans*	Gill rakers (2nd arch) Dorsal rays Thoracic vertebrae	Caudal peduncle depth Length of upper jaw Length of left pharyngeal Dorsal fin base	Caudal peduncle depth Dorsal rays Length of upper jaw Head depth at occiput
Discrimination	(98.8%)	(96.5%)	(95.3%)
G. robusta/*G. cypha*	Anal rays Caudal vertebrae Gill rakers (2nd arch)	Depth of frontal depression Anal origin to caudal base	Head depth at eye Dorsal fin base Anal fin base Pectoral to pelvic insertion
Discrimination	(95.7%)	(99.3%)	(99.3%)
G. robusta/*G. cypha*/ *G. elegans*	Gill rakers (2nd arch) Anal rays Dorsal rays Thoracic vertebrae Caudal vertebrae	Caudal peduncle depth Depth of frontal depression Anal origin to caudal base Dorsal fin base Length of upper jaw	Caudal peduncle depth Anal origin to caudal base Snout length Snout to occiput Dorsal fin base
Discrimination	(97.6%)	(96.4%)	(97.0%)

(81 of 81), and *G. cypha* and *G. elegans* were specified in 93.1% (54 of 58) and in 96.4% (27 of 28), respectively. Field characters correctly allocated all specimens in 96.3% of cases (132 of 137). Again, *G. robusta* was correctly designated in 100% (81 of 81), and *G. cypha* and *G. elegans* were classified in 96.6% (56 of 58) and in 89.3% (25 of 28), respectively. Only 2 (of 24) individuals incorrectly allocated among the three species were shared between the three data subsets.

The five field characters (Table 1) were used to derive a parametric discriminant function and to perform a canonical variate analysis. With regard to the former, the jackknife procedure failed to improve the original classification of individuals into species (five were misidenified: two *G. cypha* as *G. elegans* and three *G. elegans* as *G. cypha*). This classification was calculated to be 94% better than chance alone (kappa = 0.9417; Z = 19.463; $P < 0.00001$). A three-dimensional plot of individuals on the first three canonical axes is presented in Figure 2. Only axes 1 and 2 were significant (eigenvalue for canonical variate 1 = 8.6068 [87.4% of total variation]; eigenvalue for canonical variate 2 = 1.24 [12.6% of total variation]). When the discriminant analysis was performed with equal sample sizes (N = 28) and with the use of within-group covariance matrices, only three individuals were misrepresented (one *G. cy-*

pha was allocated to *G. elegans* and two *G. elegans* to *G. cypha*).

Classification of Juveniles and Putative Hybrids

Sixteen putative hybrids and 142 juveniles were designated to species by inserting relevant $\log_{10}$-transformed measurements corrected for overall body size into the three formulae below. An unknown specimen is allocated to the species that achieves the highest of the three scores.

Gila robusta = [(546.15·snout length) + (382.88· snout to occiput) + (966.72·dorsal base) + (752.3·caudal peduncle depth) + (1,573.58· anal origin to caudal base)] − 1,663.41.

Gila cypha = [(470.37·snout length) + (481.35· snout to occiput) + (840.49·dorsal base) + (863.59·caudal peduncle depth) + (1,441.2· anal origin to caudal base)] − 1,630.01.

Gila elegans = [(529.18·snout length) + (459.55· snout to occiput) + (820.2·dorsal base) + (941.2·caudal peduncle depth) + (1,489.33· anal origin to caudal base)] − 1,783.62.

With the use of these functions, 88% (126 of 142) of the juveniles were correctly classified. For individual species, the results were as follows: *G. robusta,* 97.2% (103 of 106); *G. cypha,* 60.0% (18 of 30), and *G. elegans,* 66.7% (4 of 6). The three misclassified *G. robusta* juveniles were allocated
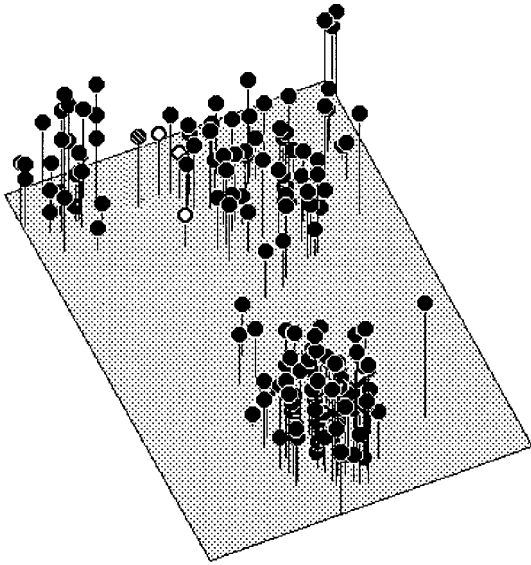
FIGURE 2.—A three-dimensional plot depicting arrangement of 167 adult *Gila robusta, G. cypha,* and *G. elegans* in canonical variate space. Three groups are apparent: *G. elegans* (left rear), *G. cypha* (right rear), and *G. robusta* (foreground). The white spheres represent three *G. elegans* classified as *G. cypha.* The single stippled sphere represents a *G. cypha* classified as *G. elegans.*

instead to *G. cypha,* and the 12 misclassified *G. cypha* were assigned to *G. robusta.* The score for one additional (correctly identified) *G. cypha* was virtually identical to that for *G. robusta.* The two misclassified *G. elegans* juveniles were assigned to *G. robusta,* and two other (correctly identified) *G. elegans* had scores virtually identical to *G. cypha* and *G. cypha*/*G. robusta,* respectively.

Among the putative hybrids, *G. robusta* × *G. cypha* was assigned 60% of the time (6 of 10) to *G. robusta* and 40% (4 of 10) to *G. cypha.* Putative *G. cypha* × *G. elegans* was assigned 50% of the time (2 of 4) to *G. cypha,* and 50% (2 of 4) to *G. elegans. Gila seminuda* was categorized as *G. robusta* in 66.7% of cases (2 of 3) and as *G. cypha* in 33.3% (1 of 3).

Discussion

Discrimination of Species

Holden (1968) and Holden and Stalnaker (1970) noted that *G. robusta* and *G. elegans* shared little morphometric similarity (81% of the former and 89% of the latter could be segregated). Each phenotype thus exhibited a high degree of within-group homogeneity, in spite of the fact that samples were taken from throughout the Colorado River basin. The five characters which best discriminated between the two species were: dorsal and anal rays, head depth, length of upper jaw, and least depth of caudal peduncle.

Our results support those of Holden and Stalnaker (1970). Of all species-pairs under evaluation, *G. robusta*/*G. elegans* was most easily separated, and by all three character sets. However, our results are more parsimonious than those of Holden and Stalnaker, in that three characters (two meristic and one morphometric) were found to be maximally discriminating.

Holden (1968) and Holden and Stalnaker (1970) noted that, although morphological relationships between *G. cypha* and *G. elegans* are unclear, hump and snout characteristics (Figure 1) are critical to their discrimination (*G. elegans* has a smooth hump and no overhanging snout; "typical" *G. cypha* has an abrupt hump and long, overhanging snout). Results from our study (Table 1) concur; *G. cypha*/*G. elegans* are indeed the most difficult of the three species-pairs to separate. *Gila cypha,* with its exaggerated nuchal morphology (Figure 1), had long been considered by fishery managers as a male *G. elegans* (reviewed in Douglas 1993).

Holden and Stalnaker (1970) argued that although *G. cypha* and *G. elegans* are separate entities, they are completely bridged by a third group that lacks the internal cohesiveness or homogeneity of the first two. Some members of the third group are more similar to *G. cypha,* and others resemble *G. elegans.* Holden and Stalnaker (1970) offered three hypotheses to account for this situation: (1) incomplete speciation with subsequent introgressive hybridization; (2) one morphologically variable species; or (3) two species, with intergrades being variants of one or the other. Smith et al. (1979) argued that the majority of intergrades in the Holden and Stalnaker (1970) study were actually *G. cypha.* The discrepancy was due to reduced precision in the original analyses when continuous data were collapsed to categorical.

In this study, meristic data (Table 1) best segregated *G. cypha* and *G. elegans.* However, meristic and morphometric subsets used characters that were not useful for field analysis. The field subset (in spite of losing three important characters) still delineated individuals 95.3%. Trophic characters were apparent in all three data subsets; this at least offers the suggestion that some form of resource partitioning may occur between these species. Depth of caudal peduncle is also a discriminating character, suggesting that *G. cypha* (at least with regards to its peduncle) is less of a "bony tail"

than *G. elegans.* It is also worth noting that depth of frontal depression (a direct measure of nuchal concavity) was not a major component of species segregation. However, a more indirect measure (head depth at occiput) was.

Gila robusta and *G. cypha* have been a focus for the majority of morphometric research accomplished on this group to date. Douglas et al. (1989) used qualitative characteristics to separate these species in the Yampa River. Kaeding et al. (1990) compared field identification at Black Rocks, Colorado, with taxonomic assignment based on a principal component analysis (PCA). In the former, the complex relationships between anal fin and caudal peduncle, eye and jaw, as well as snout, occiput, and breast–nuchal scalation, were deemed important; in the latter, the most important characters were depth of nuchal depression and caudal peduncle depth. McElroy and Douglas (1995) examined population-level differentiation of these species from eight sites in the upper Colorado River basin, and McElroy et al. (1997) differentiated the two species on four morphometric characters (eye diameter, caudal peduncle, orbit–jaw relation, and skull depression–nuchal hump).

In this study, morphometric characters (Table 1) best segregated the two species, and depth of frontal depression was an important character, which was also the case for Kaeding et al. (1990) and McElroy et al. (1997). A similar level of discrimination is maintained in the field subset, even though the former character was removed (albeit with the addition of two additional morphometric characters). However, other morphometric characters listed in Table 1 have not been previously noted as discriminatory. For example, two of four field characters (anal fin base and head depth at eye) relate more to qualitative characters depicted in Douglas et al. (1989: Figure 2) than to any others. Two remaining field characters (dorsal fin base and pectoral insertion to pelvic insertion) have not been previously identified as important. Their designation supports Douglas et al. (1989), who argued for application of new approaches and new characters in resolving this complex problem.

Smith et al. (1979) evaluated phenotypic relationships among all three study species using PCA and noted that characters best contributing to their separation were dorsal and anal rays, gill raker and vertebral number, and depth and length of caudal peduncle. Those meristic characters indicated by Smith et al. (1979) also discriminated well in our analyses (Table 1). In addition, caudal peduncle depth was an influential morphometric character,

as were several others not mentioned by these authors. Discriminating field characters pertained to the peduncle, its relation to the anal fin, the snout, its relation to the occiput, and the dorsal fin base. All three data sets discriminated greater than 95%.

It is clear from this study that a variety of characters differ significantly among these species, and various combinations of characters provide excellent discrimination. The high kappa value ($P < 0.00001$) and failure of the jackknife to improve the original segregation underscore this point. Furthermore, misclassified individuals, both within each species-pair and among all three species, were rarely shared among the three data subsets, again suggesting that a variety of characters adequately discriminate. We thus reject the hypothesis that the three species are phenotypically indistinguishable.

It is also clear that *G. elegans* is the most difficult of the three to assign to group. Of four misidentified individuals in the discriminant analysis with field data, three were *G. elegans* (Figure 2). Similarly, when sample sizes were equilibrated among species and within-group covariances were used in the analysis, two (of three) misidentified individuals were *G. elegans.*

Assignment of Juveniles and Putative Hybrids to Species

Overall, assignment of juveniles to species seemed reasonable, with 88% correctly allocated. However, this percentage is misleading. The preponderance of juveniles were *G. robusta* ($N = 106$; 75%), and these were correctly assigned at greater than 97%. Correct assignment of juveniles to *G. cypha* and *G. elegans* were only 60% and 66%, respectively. Furthermore, if one considers that several correct assignments of juveniles to these species were actually borderline with respect to score, then the number of unambiguous assignments is even weaker. This suggests that although field characters (Table 1) may be satisfactory for designating juvenile *G. robusta* to species, they appear marginal at best in assigning juvenile *G. cypha* and *G. elegans.* Quite possibly, the occiput and nuchal hump have not as yet developed adequately enough to serve as the diagnostic tool these structures provide in adults (Table 1). Given these mixed results, we cannot reject our second hypothesis, that adult characters also segregate juveniles. However, this does not mean that juveniles of *G. cypha* and *G. elegans* cannot be differentiated phenotypically. Composites of several different quantitative characters may instead be useful;

these, in turn, may change during ontogeny so as to become less discriminating. Thus, application of a few discriminating adult characteristics (as in Table 1) may not be reliable enough to segregate juveniles.

With regard to our third hypothesis, putative hybrids were generally assigned to either one or the other of their parental forms. Hybrids included specimens of *G. seminuda* (one of which was assigned to *G. cypha* rather than *G. elegans*). If the premise that these are indeed ''hybrids'' is acceptable, then our results appear intuitive and the hypothesis of hybrid intermediacy cannot be rejected (in spite of the fact that DeMarais et al. [1992] clearly demonstrated that *G. seminuda* was phenotypically more like *G. robusta* than *G. elegans*).

Choice of Characters and the ''Art of Seeing Well''

When meristic data were evaluated, dorsal or anal rays appeared diagnostic in virtually every species comparison (Table 1). Yet, surprisingly, these characters were of little assistance in field application, being discriminatory in only one comparison. We also note in this context that vertebral counts and numbers of gill rakers were equally as discriminating as the former.

However, morphological data provided greatest discriminating power in segregating species. This may be because morphometric characters best describe shape and shape change, and these aspects are paramount in discriminating among big-river *Gila.* Morphological characters also formed 71% of the total number of characters used in our analyses, so their ascendancy may also be related to their numerical superiority. Nevertheless, it is clear that the three *Gila* in this report can easily be assigned to species, based upon a variety of counts and measurements. So, in a sense, we are troubled that there is such a history of confusion among fishery managers with regard to this species complex, particularly given results in Table 1 and Figure 2. We can only conclude that this bewilderment is a manifestation of those perspectives offered by Holden (1991, see above). Douglas et al. (1989) touched upon a similar point when they stated

> From our perspective, some confusion shrouding identification of problematic *Gila* hinges upon apparent misunderstanding of the populational nature of natural selection (a statistical concept). The conservative nature of fisheries science, with reliance on an idealized morphological archetype to represent a spe-

cies, contributes substantially to this problem. Variation within and among populations and species must be recognized as a natural phenomenon amenable to statistical analysis (as herein), rather than as an infrequent occurrence to be either redefined or dismissed.

The failure of fishery managers to accept this premise on a broad basis, coupled with an historical bias against native fishes in western North America, has both introduced and perpetuated the confusion surrounding this group of fishes. In our analyses, it appears as if answers to species distinctness hinge upon selection of characters and adequacy of analyses (Douglas 1993), coupled with ''the art of seeing well,'' which was defined by Rafinesque (1820) as ''the art . . . of noticing and distinguishing with accuracy the objects which we perceive . . . [It] is a high faculty of the mind, unfolded in a few individuals, and despised by those who can neither acquire it nor appreciate its results.''

References

Bosley, C. E. 1960. Pre-impoundment study of the Flaming Gorge Reservoir. Wyoming Game and Fish Commission Technical Report 9:1–81.

DeMarais, B. D., T. E. Dowling, M. E. Douglas, W. L. Minckley, and P. C. Marsh. 1992. Origin of *Gila seminuda* (Teleostei: Cyprinidae) through introgressive hybridization: implications for evolution and conservation. Proceedings of the National Academy of Sciences 89:2747–2751.

Douglas, M. E. 1993. An analysis of sexual dimorphism in an endangered cyprinid fish, *Gila cypha* Miller, using video image analysis. Copeia 1993:334–343.

Douglas, M. E., W. L. Minckley, and H. M. Tyus. 1989. Qualitative characters, identification of Colorado River chubs (Cyprinidae: genus *Gila*) and the ''art of seeing well.'' Copeia 1989:653–662.

Douglas, M. E., G. D. Schnell, and D. J. Hough. 1984. Differentiation between inshore and offshore spotted dolphins from the eastern tropical Pacific Ocean. Journal of Mammalogy 65:375–387.

Douglas, M. E., G. D. Schnell, D. J. Hough, and W. F. Perrin. 1992. Geographic variation in cranial morphology of spinner dolphins *Stenella longirostris* in the eastern tropical Pacific Ocean. Fishery Bulletin 90:54–76.

Dowling, T. E., and B. D. DeMarais. 1993. Evolutionary significance of introgressive hybridization in cyprinid fishes. Nature (London) 362:444–446.

Fradkin, P. L. 1984. A river no more—the Colorado River and the west. University of Arizona Press, Tucson.

Holden, P. B. 1968. Systematic studies of the genus *Gila* (Cyprinidae) of the Colorado River basin. Master's thesis. Utah State University, Logan.

Holden, P. B. 1991. Ghosts of the Green River: impacts of Green River poisoning on management of native fishes. Pages 43–54 *in* W. L. Minckley and J. E. Deacon, editors. Battle against extinction: native fish management in the American west. University of Arizona Press, Tucson.

Holden, P. B., and C. B. Stalnaker. 1970. Systematic studies of the cyprinid genus *Gila* in the upper Colorado River basin. Copeia 1970:409–420.

Hubbs, C. L., L. C. Hubbs, and R. E. Johnson. 1943. Hybridization in nature between species of catostomid fishes. Contributions from the Laboratory of Vertebrate Biology of the University of Michigan 22:1–76.

Jackson, D. A., and K. M. Somers. 1991. The spectre of 'spurious' correlations. Oecologia 86:147–151.

Kaeding, L. R., B. D. Burdick, P. A. Shrader, and C. W. McAda. 1990. Temporal and spatial relations between the spawning of humpback chub and roundtail chub in the upper Colorado River. Transactions of the American Fisheries Society 119:135–144.

McDonald, D. B., and P. A. Dotson. 1960. Fishery investigations of the Glen Canyon and Flaming Gorge impoundment areas. Utah Department of Fish and Game Information Bulletin 60(3):1–70.

McElroy, D. M., and M. E. Douglas. 1995. Patterns of morphological variation among endangered populations of *Gila robusta* and *G. cypha* (Teleostei: Cyprinidae) in the upper Colorado River basin. Copeia 1995:636–649.

McElroy, D. M., J. A. Shoemaker, and M. E. Douglas. 1997. Discriminating *Gila robusta* and *Gila cypha*: risk assessment and the Endangered Species Act. Ecological Applications 7:958–967.

Miller, R. R. 1945. A new cyprinid fish from southern Arizona, and Sonora, Mexico, with description of a new subgenus of *Gila* and a review of related species. Copeia 1945:104–110.

Miller, R. R. 1946. *Gila cypha,* a remarkable new species of cyprinid fish from the Colorado River in Grand Canyon, Arizona. Journal of the Washington Academy of Sciences 36:409–415.

Minckley, C. O. 1996. Observations on the biology of the humpback chub in the Colorado River basin 1908–1990. Doctoral dissertation. Northern Arizona University, Flagstaff.

Rafinesque, C. S. 1820. Ichthyologia Ohioensis, or natural history of the fishes inhabiting the river and its tributary streams, preceded by a physical description of the Ohio and its branches. W. G. Hunt, Lexington, Kentucky. (Reprint of Call 1889.)

SAS Institute. 1989. SAS/STAT user's guide version 6, volume 1. SAS, Cary, North Carolina.

Schnell, G. D., G. L. Worthen, and M. E. Douglas. 1985. Morphometric assessment of sexual dimorphism in skeletal elements of California gulls. Condor 87:484–493.

Smith, G. R. 1960. Annotated list of fishes of the Flaming Gorge Reservoir basin, 1959. Pages 195–199 *in* A. M. Woodbury, editor. Ecological studies of the flora and fauna of Flaming Gorge Reservoir basin, Utah and Wyoming. University of Utah Anthropological Paper 48.

Smith, G. R., R. R. Miller, and W. D. Sable. 1979. Species relationships among fishes of the genus *Gila* in the upper Colorado River drainage. Pages 613–623 *in* R. M. Linn, editor. Proceedings of the first conference on scientific research in the National Parks. U.S. National Park Service Transactions and Proceedings Series 5:613–623.

Sofield, R. K., M. E. Douglas, E. J. Hansens, and R. C. Vrijenhoek. 1984. Diagnosis and detection of cryptic species: the *Tabanus nigrovittatus* complex (Diptera: Tabanidae) in coastal New Jersey. Annals of the Entomological Society of America 77:587–591.

Titus, K., J. A. Mosher, and B. K. Williams. 1984. Chance-corrected classification for use in discriminant analysis: ecological applications. American Midland Naturalist 111:1–7.

Vanicek, C. D., and R. H. Kramer. 1969. Life history of the Colorado squawfish, *Ptychocheilus lucius,* and the Colorado chub, *Gila robusta,* in the Green River in Dinosaur National Monument, 1964–1966. Transactions of the American Fisheries Society 98:193–208.

Appendixes follow

Appendix 1: Meristic and Morphometric Data of *Gila* spp.

TABLE A1.—Meristic and morphometric variables for three species of *Gila* studied. Data are untransformed means (SDs in parentheses); sample sizes are given for each species.

Variable	G. robusta (N = 81)	G. cypha (N = 58)	G. elegans (N = 28)
Meristic character counts			
Dorsal rays	9.00 (0.00)	9.22 (0.42)	10.00 (0.39)
Anal rays	9.15 (0.36)	10.07 (0.32)	10.36 (0.49)
Lateral line scales	84.99 (5.18)	82.12 (3.78)	85.36 (5.53)
Gill rakers (2nd arch)[a]	13.52 (1.01)	15.26 (1.05)	18.29 (1.53)
1st pharyngeal teeth (left posterior limb)[a]	2.07 (0.41)	1.97 (0.18)	2.07 (0.38)
2nd pharyngeal teeth (left posterior limb)[a]	4.93 (0.26)	4.97 (0.18)	4.96 (0.19)
3rd pharyngeal teeth (left posterior limb)[a]	4.02 (0.16)	3.98 (0.13)	4.04 (0.19)
4th pharyngeal teeth (left posterior limb)[a]	2.04 (0.37)	1.91 (0.34)	2.04 (0.19)
Thoracic vertebrae[a]	19.91 (0.61)	20.00 (0.73)	21.79 (0.79)
Caudal vertebrae[a]	22.11 (0.88)	23.24 (0.92)	23.07 (0.66)
Morphometric character length (mm)			
Standard length	250.50 (35.8)	253.09 (24.9)	280.23 (28.6)
Head length	66.19 (10.3)	60.93 (5.42)	63.79 (5.57)
Eye diameter	6.85 (1.76)	7.02 (0.81)	8.47 (0.89)
Snout length	21.81 (3.79)	21.20 (2.52)	19.41 (2.22)
Preanal length	168.73 (26.5)	158.19 (15.6)	176.80 (17.6)
Head depth through eye	25.68 (3.76)	20.33 (1.59)	20.67 (2.09)
Head depth at occiput	37.71 (5.76)	32.72 (2.50)	36.47 (4.02)
Interorbital width (bony)	21.95 (3.79)	21.29 (1.99)	21.48 (2.16)
Tip of snout to occiput	48.59 (8.11)	41.06 (4.21)	45.05 (4.45)
Dorsal fin base	32.42 (4.65)	36.73 (3.59)	39.64 (4.15)
Anal fin base	30.15 (4.32)	36.13 (4.23)	40.30 (4.94)
Predorsal length	129.71 (19.9)	125.06 (11.5)	134.08 (14.4)
Pectoral fin length	47.98 (7.25)	51.15 (5.48)	51.27 (5.85)
Pelvic fin length	38.33 (5.69)	40.68 (4.42)	42.22 (4.51)
Length of upper jaw	23.32 (4.10)	20.50 (2.61)	18.94 (2.16)
Mouth width	19.53 (3.49)	18.82 (2.79)	18.41 (2.22)
Body depth at pectoral fin insertion	46.87 (7.16)	49.28 (5.92)	47.96 (6.72)
Depth of caudal peduncle	15.73 (2.26)	13.28 (1.27)	11.65 (1.54)
Anal fin origin to caudal base	88.79 (12.6)	103.58 (12.3)	112.98 (12.4)
Length of left pharyngeal bone[a]	21.85 (3.90)	18.68 (1.88)	20.72 (1.89)
Width of left pharyngeal bone[a]	9.99 (4.21)	8.20 (3.57)	7.75 (3.52)
Length of posterior pharyngeal limb[a]	11.46 (0.36)	10.74 (0.34)	13.58 (0.37)
Length of anterior pharyngeal limb[a]	12.59 (0.47)	10.96 (0.40)	12.10 (0.37)
Pectoral insertion to pelvic insertion	59.97 (9.26)	51.93 (5.24)	56.96 (8.11)
Depth of skull's frontal depression[a]	1.10 (0.65)	4.92 (1.48)	3.07 (1.00)

[a] Excluded from field data set.

Appendix 2: Sources of *Gila* Specimens

Sources and numbers (*N*) of *Gila* specimens examined for this study are listed below (Acc. = accession; uncatlg. = uncatalogued). Institutional abbreviations are as follows: ASU, Arizona State University; CDW, Colorado Division of Wildlife; CSU, Colorado State University; FCM, Field Columbia Museum (Field Museum of Natural History, Chicago); MNA, Museum of Northern Arizona; NERC, National Ecology Research Center; NMGF, New Mexico Game and Fish; TU, Tulane University; UCO, University of Colorado; UMIN, University of Minnesota; UMMZ, University of Michigan Museum of Zoology; UNLV, University of Nevada in Las Vegas; UNM, University of New Mexico; USNM, National Museum of Natural History.

Gila robusta adults (*N* = 81): UNM 3236 (6); UNM 3076 (17); UNLV 33 (1); UMMZ 142538 (1); UMMZ Acc.1978-I:17 (17) [NERC uncatlg. (17)]; UMMZ Acc.1976-XI: 23 (4) [NMGF uncatlg. (2); CDW uncatlg. (2)]; UNM 2619 (1); UNM 1746 (1); UMMZ 182546 (25); UMMZ 160648 (1); UMMZ 162335 (1); UMMZ 179580 (1); UMMZ Acc.1964-II:14 (2) [CSU uncatlg. (2)]; UMMZ 182546 (1); USNM 246 (1); TU 95847 (1).

Gila robusta juveniles (*N* = 106): UNM 3236 (2); UNM 3076 (9); UNLV 7 (5); UNLV 15 (4); UNLV 22 (4); UNLV 25 (4); UNLV 10 (2); UNLV 33 (8); UNLV 16 (3); UNLV 27 (10); ASU 4068 (8); UMMZ 142543 (10); UMMZ 142538 (9); UMMZ 167553 (3); UMMZ Acc.1978-I:17 (3) [NERC uncatlg. (3)]; UMMZ 161782 (1); UMMZ Acc.1968-IX:1 (1) [ASU 2490 (1)]; UNM 1735 (1); UNM 1741 (1); UNM 3840 (1); UNM 1742 (3); UNM 2619 (1); UNM 1746 (1); UNM 3723 (5); ASU 2490 (1); UMMZ 117835 (5); UMMZ 117834 (1).

Gila cypha adults (*N* = 58): UMMZ Acc.1978-I:17 (9) [NERC uncatlg. (9)]; ASU 7035 (3); ASU 7049 (1); ASU 7036 (1); UMMZ Acc.1968-IX:1 (9) [ASU 2487 (9)]; UMMZ 182544 (2); UMMZ 160647 (2); UMMZ 162336 (2); UMIN 16353 (2); UMMZ 179577 (1); UCO 6044 (1); UCO 6043 (2); UCO 6042 (2); UMMZ Acc.1964-II:14 (1) [CSU uncatlg. (1)]; UMMZ 181281 (2); USNM 199472 (1); USNM 189209 (1); MNA 2740 (3); USNM 131839 (1); UMMZ 189228 (1); UMMZ 191632 (1); ASU 4753 (1); UMMZ Acc.1968-IX:1 (1) [ASU 2487 (1)]; FCM 3190 (1); FCM 2593 (1); FCM 3267 (1); FCM 2531 (1); FCM 2592 (1); FCM X (1); FCM 2767 (1); FCM 2766 (1).

Gila cypha juveniles (*N* = 30): UMMZ 198453 (17); UMMZ 189209 (2); UMMZ 182074 (2); UMMZ 182416 (1); UMMZ 180090 (8).

Gila elegans adults (*N* = 28): ASU 7035 (1); ASU 5143 (1); ASU 7048 (1); ASU 7036 (1); ASU 7051 (1); UMMZ 181287 (1); UMMZ 182545 (2); UMIN 19019 (2); UMMZ 179581 (1); UMMZ Acc.1964-II:14 (12) [CSU uncatlg. (12)]; UMMZ 181282 (2); UMMZ 181287 (2); USNM 251 (1).

Gila elegans juveniles (*N* = 6): UMMZ 94865 (6).

Gila robusta × *G. cypha* (*N* = 10): UMMZ 182546 (3); UMMZ Acc.1968-IX:1 (6) [ASU 2490 (6)]; UMMZ Acc.1978-I:17 (1) [NERC uncatlg. (1)]

Gila cypha × *G. elegans* (*N* = 4): FCM 2593 (1); FCM 2831 (1); FCM 2845 (1); UMMZ 162336 (1).

Gila seminuda (*N* = 3): UMMZ 141666 (3).