

DISCRIMINATING *GILA ROBUSTA* AND *GILA CYPHA*: RISK ASSESSMENT AND THE ENDANGERED SPECIES ACT

DOUGLAS M. McELROY,¹ JULIE A. SHOEMAKER,¹ AND MICHAEL E. DOUGLAS²

¹Department of Biology, Western Kentucky University, Bowling Green, Kentucky 42101 USA

²Department of Zoology and Museum, Arizona State University, Tempe, Arizona 85287-1501 USA

Abstract. Several sympatric Colorado River basin cyprinid fishes of the genus *Gila* are federally protected, yet difficulty in identifying individuals to species has limited recovery efforts. Using five characters easily scored in the field, we quantified morphological variation in *G. robusta* and *G. cypha* from eight localities, comparing discriminatory power of these data to that of a previous multivariate truss analysis of the same specimens. Significant between-species differences existed in four characters; three displayed patterns consistent with typological differences between species. Success of post hoc identification of specimens exceeded 70% for these three characters and increased to >82% by incorporating variables into a discriminant function. Nevertheless, potentially diagnostic features were relatively uncorrelated within individuals, and little congruence existed between different characters. Multivariate analyses clearly discriminated between sympatric species pairs despite considerable variation among localities. None of these analyses generated patterns of phenetic relationships approximating those suggested by the prior truss analysis. Whereas field characters are broadly diagnostic, classification of a significant number of individuals from any sample will likely remain problematic. We explore the implications of this problem within the context of the “producer’s vs. consumer’s risk” gambit, arguing that management strategies for *Gila* should accept the “producer’s risk” and define endangered species broadly. We also advocate use of the risk gambit for the general problem of evaluating taxonomic distinctness of endangered taxa.

Key words: Colorado River; conservation and management; discriminant analysis; endangered species; *Gila*; hybridization; morphology; producer’s vs. consumer’s risk; taxonomic distinctness; video image analysis.

INTRODUCTION

In affording protection to “any distinct population segment of any species of vertebrate fish or wildlife,” the Endangered Species Act (ESA) of 1973 established a powerful (Carroll et al. 1996) yet contentious (O’Brien 1994) criterion for the recovery of imperiled taxa. The need to demonstrate taxonomic distinctness of biological units eligible for protection has generated controversy within the biological community (cf. Wayne and Jenks 1991, Dowling et al. 1992a, b, Wayne 1992) as well as legal challenges to management plans (cf. O’Brien and Mayr 1991, Avise 1994), and remains a primary focus of both debate (Franklin 1993, Eisner et al. 1995) and effort among conservation biologists and managers. In fact, a revised policy dealing with the treatment of hybrids is currently being considered by the Department of the Interior (U.S. Fish and Wildlife Service [USFWS] 1996). Several case histories (e.g., Florida panther [*Felis concolor coryi*], red wolf [*Canis rufus*], Pacific salmon [genus *Oncorhynchus*], Northern Spotted Owl [*Strix caurina occidentalis*]) have been used to highlight difficulties associated with

interpreting “distinctness” under the ESA. Though less well publicized, fishes of the genus *Gila* endemic to the American Southwest present an equally complex and similarly urgent example of the conflict between biological reality and legislative necessity.

The upper Colorado River basin cyprinid, *Gila cypha*, is one of the most endangered fish species in the world (USFWS 1985, 1987). Only seven isolated populations are extant (Douglas and Marsh 1996); of these, only two or three are thought to be self-sustaining (R. Valdez, Bio/West, Logan, Utah, *personal communication*). The status of at least two other members of the *G. robusta* species complex is similarly tenuous; *G. elegans* may be extinct in the wild (Douglas et al. 1989, Kaeding et al. 1990), and *G. robusta* is declining throughout its range (W. L. Minckley, *personal communication*). The latter is a candidate species for listing under the ESA (USFWS 1994).

Threatened or endangered status entitles these species to protection under state and/or federal statutes. However, high morphological variability within and among species (Smith et al. 1979, Douglas et al. 1989, McElroy and Douglas 1995) and data suggesting a recurrent pattern of hybridization and gene exchange among taxa over evolutionary time (DeMarais et al. 1992, Dowling and DeMarais 1993) have confounded

Manuscript received 25 September 1995; revised 20 August 1996; accepted 19 September 1996; final version received 4 November 1996.

attempts to designate populations for protection (Douglas et al. 1989). In particular, difficulty in identifying individuals to species has limited the ability to meet minimum standards for invoking ESA protection. Significant effort has thus been expended in searching for ways to rapidly, reliably, and nondestructively identify individuals in the field.

Douglas et al. (1989) used qualitative field characters to separate putative *G. robusta* and *G. cypha* from the upper Yampa River (Douglas et al. 1989), whereas Kaeding et al. (1990) compared field identification of these species from Black Rocks (see McElroy and Douglas 1995) to taxonomic assignments based on quantitative morphometric analyses. Both succeeded in separating species based on a limited number of characters; however, methodological differences prevent direct comparisons of patterns of variation between localities. McElroy and Douglas (1995) applied video image acquisition methods (Douglas 1993) and multivariate morphometrics to analysis of variation within and among populations of *G. robusta* and *G. cypha* from eight localities. That study provided extremely fine resolution of differences among populations, as well as the ability to assess morphological consistency of species across localities; however, the required technical and analytical sophistication makes such an approach impractical for field identification of specimens.

Here we integrate the field-oriented approach of Douglas et al. (1989) and Kaeding et al. (1990) with the comparative power of McElroy and Douglas (1995). Using the same specimens as McElroy and Douglas (1995), we examine patterns of variation in five morphological characters easily scored in the field. We directly address three questions: (1) How is variation in each character partitioned among populations and species, and which if any are useful in discriminating between sympatric *G. robusta* and *G. cypha*? (2) Are patterns of variation between species consistent among localities and characters? (3) Do any characters show patterns of phenetic relationships similar to those described by McElroy and Douglas (1995), thus suggesting their efficacy as reliable markers of population distinctiveness? At this level, our objective is to provide further insight or guidance to managers charged with developing and instituting recovery plans for species of Colorado basin *Gila*.

We also consider the difficulties inherent in, and the lessons learned from, studies of upper basin *Gila* taxonomy within the context of a more general problem in conservation and management: Given that identification of individual specimens of endangered species may be problematic (due to a lack of diagnostic characters and/or the existence of hybridization with other, nonthreatened taxa), what is the most expedient way to define biological units for protection? We invoke a classic risk assessment paradigm (Sokal and Rohlf 1981) as a metaphor for this problem, and discuss the implications of employing two alternative strategies in

making management decisions. We relate our perspectives to those derived from studies of other problematic taxa, and propose a means by which this risk gambit may be incorporated into recovery priority analysis.

METHODS

Choice of characters

G. cypha has been contrasted typologically with *G. robusta* on the basis of (among other features) a prominent anterodorsal hump and markedly concave skull, relatively thinner caudal peduncle, small eyes, and an essentially horizontal mouth (Miller 1946, Minckley 1973, 1991, Douglas et al. 1989). These characteristics have been interpreted as adaptations to the high current regimes and silt loads typical of its whitewater habitat in the preimpoundment Colorado River (Miller 1946, Minckley 1973; but see Kaeding et al. 1990). In addition, *G. cypha* has been characterized as having a larger gape than does *G. robusta* (Douglas et al. 1989). Such features are largely qualitative and subsume multiple aspects of shape; at the same time, they are also readily quantifiable using simple geometric manipulations, and so may be measured with reasonable accuracy in both the laboratory and field. We developed five continuous measures to describe these potentially diagnostic characters (Table 1).

Data collection

Morphological data were collected from video images of 363 adult *G. robusta* ($n = 215$) and *G. cypha* ($n = 148$) collected by McElroy and Douglas (1995) from eight localities in the upper Colorado Basin and the Grand Canyon (see McElroy and Douglas 1995). Field sampling and image acquisition methods are described in Douglas (1993) and McElroy and Douglas (1995). For each specimen, coordinates of anatomical landmarks digitized directly from videotape were used to geometrically extract five characters (Table 1) using MorphoSys (version 1.29 OFG, Meacham 1993). All measurements except mouth angle (MA) were expressed relative to a scale bar present on each image, in units of millimeters. MA was expressed in degrees of clockwise rotation from a horizontal line from the tip of the snout through the caudal fin fork. Total length (TL) was measured directly from individual specimens during videotaping, and recorded both in field notes and on the videotape itself.

Identifications of specimens to species derive from the earlier study of McElroy and Douglas (1995). Using a much larger (and largely independent) morphometric data set, they were able to unambiguously classify all 363 fish. While genetic data theoretically constitute a more objective criterion, in reality such characters are likely to be as confused as is morphology (Dowling and DeMarais 1993, McElroy and Douglas 1995). Beyond that, our approach is appropriate to the question being asked; that is, can we identify simple field mea-

tures that capture the information contained in a more comprehensive character set? This is essential, as morphological characterizations remain the primary criteria for establishing taxonomic distinctness under the ESA (O'Brien and Mayr 1991).

Data analysis

Data for eye diameter (ED), caudal peduncle (CP), orbit-jaw relationship (OJ), and skull depression–nuchal hump (SD) (Table 1) were size-adjusted by dividing raw measurements by total length for that individual. Due to discrepancies in recording of TL between field notes and video, 16 individuals (11 *G. robusta*, 5 *G. cypha*) were excluded. We justify the use of ratio standardization in three ways. First, Rising and Somers (1989) have demonstrated that univariate measures of overall size that are relatively independent of aging and condition effects closely approximate multivariate measures; we argue that TL is relatively insensitive to such confounding factors. Second, we rejected the null hypothesis of collinearity and zero-intercept between ratio components in only 1 of 52 comparisons based on Bonferroni-adjusted criteria (ED vs. TL in Yampa River *G. robusta*); when such null criteria are met, ratios correctly remove effects of overall size (Jackson and Somers 1991). Finally (and most importantly), our objective is to provide field workers with a way to classify *Gila* specimens; multivariate size correction defeats this purpose. MA was not size adjusted, as it is an angular measure geometrically independent of size.

These data were tested for deviations from normality by species and population ($N_{groups} = 13$) using the Komogorov-Smirnov/Lilliefors algorithm in SYSTAT (version 5 for Windows, SYSTAT 1992). Two of 65 character \times population comparisons (3.1%) deviated significantly from normality based on Bonferroni-adjusted criteria. Because of the limited evidence for non-normality, no further transformations were applied.

Each size-adjusted character and MA was tested for significant differences between species (pooled across populations) using ANOVA. Levels of significance were assessed based on Bonferroni-adjusted criteria. The discriminatory power of characters was evaluated through post hoc assignment of individual specimens to species based on their relative deviations from the means of each of the two species. The proportion of correct classifications was taken as an indication of discriminatory power.

Multivariate morphological differentiation between *G. robusta* and *G. cypha* was examined through canonical discriminant analysis of characters displaying significant univariate *F* tests using SYSTAT. Predicted group membership for each specimen was estimated a posteriori based on its generalized squared Mahalanobis distance from the centroid of each group. Classification error rates were used as a measure of dis-

TABLE 1. Description of morphometric characters employed. Character values were obtained by geometric computations based on landmarks digitized by McElroy and Douglas (1995) using MorphoSys (Meacham 1992).

Character	Acronym	Description
Eye diameter	ED	Horizontal diameter of the orbit as aligned with the long body axis; larger values indicate greater eye size.
Caudal peduncle†	CP	Vertical distance at which a line projected along the base of the anal fin intersects above (positive values) or below (negative values) the posterior-dorsal margin of the caudal peduncle; larger negative values indicate a thicker peduncle.
Orbit–jaw relation†	OJ	Horizontal distance that the mouth corner lies anterior (positive values) or posterior (negative values) to the center of the eye; larger positive values indicate a smaller gape.
Skull depression/nuchal hump†,‡	SD	Euclidean distance of a line from the occiput to a point along a straight line connecting the dorsal edge of the body vertical of the pectoral origin and a point halfway between the tip of the snout and a point on dorsal edge of the body vertical of the orbit center, such that the two lines are perpendicular; larger positive distances indicate greater concavity of the skull and development of a nuchal hump.
Mouth angle	MA	The angle in degrees of clockwise rotation formed by the line drawn through the tip of the snout (vertex) and the mouth corner relative to a horizontal line from the snout through the caudal fin fork; smaller angles correspond to more horizontal mouth placement.

† Similar to character(s) used by Douglas et al. (1989).

‡ Similar to character(s) used by Smith et al. (1979).

criminatory power of the estimated functions for samples at hand.

Patterns of variation at the generic level were examined by grouping individuals by population and species ($N_{groups} = 13$). Among-group differences for each character were tested for significance using Tukey's HSD multiple comparison test, with the Tukey-Kramer

TABLE 2. Comparison of *Gila robusta* and *G. cypha* collected from eight localities in the upper Colorado River basin for five size-adjusted field characters.

Charac- ter	<i>G. robusta</i>	<i>G. cypha</i>	<i>F</i>	<i>P</i>
ED	0.0314 ± 0.0002	0.0281 ± 0.0003	80.439	<0.001
CP	-0.0214 ± 0.0012	0.0040 ± 0.0015	180.934	<0.001
OJ	0.0132 ± 0.0003	0.0152 ± 0.0004	15.922	<0.001
SD	0.0009 ± 0.0003	0.0062 ± 0.0004	123.368	<0.001
MA	42.513 ± 0.394	42.563 ± 0.492	0.304	0.582

Note: Values given are mean ± 1 SE. Tests for significant differences between species were conducted using ANOVA; degrees of freedom for all tests are 1, 345. See Table 1 for a description of character acronyms.

adjustment for unequal sample sizes among cells; this procedure protects the Type I error rate within tests (SYSTAT 1992). Multivariate differences were assessed using canonical discriminant analysis as described above.

Hierarchical relationships among groups for each character were visualized through UPGMA clustering of absolute values of pairwise differences between group means using NTSYS-pc (version 1.80, Rohlf 1993). For the multivariate analysis, generalized squared Mahalanobis distances between group means were clustered using both complete and single linkage methods, in addition to UPGMA; the robustness of resulting clusters was evaluated by computing the strict consensus of single and complete linkage trees (Rohlf 1993). The matrix correlation between each of the six pairwise distance matrices and a corresponding matrix derived from a 56-character truss analysis of the same specimens (McElroy and Douglas 1995) was estimated using a Mantel test (Mantel 1967, Rohlf 1993), with significance based on 500 permutations of the truss-derived matrix, and assessed using Bonferroni-adjusted criteria. The Mantel comparisons provide a test of congruence between phenetic relationships suggested by field characters with those derived from the earlier, more extensive multivariate analysis (McElroy and Douglas 1995). The limits of congruence were further

evaluated by producing a strict consensus of UPGMA topologies derived from the truss matrix with each of the six distance matrices generated herein.

RESULTS

Between-species differences

ED, CP, OJ, and SD differed significantly between species, while the comparison involving MA was non-significant (Table 2). Caudal peduncle depth and skull depression/nuchal hump explained the highest proportion of variation between species. Generally, *G. cypha* was characterized by having significantly smaller eyes, thinner caudal peduncle, and more pronounced anterodorsal hump and concavity of the skull than the *G. robusta* sample. In contrast to prior characterizations, however, we found the placement of the mouth corner to be significantly more anterior of the orbit in *G. cypha* than in *G. robusta*. In both species, the angle of the mouth was relatively acute (grand mean ± SE = 42.53° ± 0.31°), and could not be used to differentiate species.

Discriminatory power of three (ED, CP, SD) of the four potentially diagnostic features was >70%, and approached 80% accuracy in *G. robusta* for two characters (CP, SD; Table 3). In all cases, specimens of *G. robusta* could be assigned to species with a higher degree of confidence than could individual *G. cypha*.

Canonical discriminant analyses revealed significant multivariate differences in morphology between species (Wilks' lambda = 0.550, $F_{4,342} = 69.931$, $P < 0.001$). The discriminant function providing the best separation of *G. robusta* and *G. cypha* was estimated to be:

$$Z = -0.300(\text{ED}) + 0.630(\text{CP}) \\ + 0.194(\text{OJ}) + 0.438(\text{SD})$$

with positive values of *Z* corresponding to *G. cypha* and negative values to *G. robusta* samples. Individuals could be classified with nearly 82% accuracy (Table 3), though discriminatory power of the function was not uniform among populations (Table 4). Classification efficiency was markedly lower in samples from Cataract and Desolation canyons (pooled discriminatory power = 42%). Error rates also were relatively high for both species in Yampa River samples. Cataract and Desolation canyon fish influenced overall classi-

TABLE 3. Proportion of correct post hoc classifications of *Gila robusta* vs. *G. cypha* pooled across all eight localities for each of the four diagnostic size-adjusted field characters and the four-character discriminant function.

Charac- ter(s)	<i>G. robusta</i>	<i>G. cypha</i>	Overall
ED	0.74 (150/204)	0.65 (93/143)	0.70 (243/347)
CP	0.80 (164/204)	0.70 (100/143)	0.76 (254/347)
OJ	0.61 (125/204)	0.57 (81/143)	0.59 (206/347)
SD	0.79 (161/204)	0.68 (97/143)	0.74 (258/347)
DFA	0.83 (169/204)	0.80 (115/143)	0.82 (284/347)

Note: For each single character, specimens were assigned to the species from which the deviation of their individual score to the group mean was minimal. For the discriminant function analysis (DFA), specimens were classified based on their generalized squared Mahalanobis distance from the centroid of each group. Values in parentheses represent the number of correct classifications/total number of individuals of that group classified.

TABLE 4. Classification efficiency of the discriminant function between *Gila robusta* and *G. cypha*, separated by population.

Locality	<i>G. robusta</i>	<i>G. cypha</i>	Overall
Black Rocks (B)	1.00 (19/19)	0.80 (20/25)	0.88 (39/44)
Cataract Canyon (C)	0.83 (5/6)	0.55 (6/11)	0.65 (11/17)
Desolation Canyon (D)	0.22 (5/23)	0.45 (9/20)	0.33 (14/4)
Westwater Canyon (W)	0.93 (51/55)	0.93 (51/55)	0.93 (102/110)
Yampa River (Y)	0.94 (61/65)	0.60 (3/5)	0.91 (64/70)
Debeque (Q)	0.85 (17/20)
Rifle (R)	0.69 (11/16)
Grand Canyon (G)	...	0.96 (26/27)	...

Note: Specimens used to compute the discriminant function were assigned to species a posteriori based on their generalized squared Mahalanobis distance from the centroid of each group. Numbers in parentheses represent the number of correct classifications/total number of individuals of that group classified.

fication success; excluding these samples increased overall discriminatory success to 90%. By contrast, discrimination was not biased upwards by presence of Grand Canyon, Debeque, and Rifle samples (where only one species occurs); excluding these groups reduced discriminatory power to only 81%. These estimates of classificatory success are based on assignment of the same specimens used to compute the discriminant function; as such, they likely represent maximum values one could expect when classifying new individuals.

Differences among populations

All five morphometric characters revealed some differentiation among sympatric and allopatric populations; between 18 (OJ) and 35 (CP) of 78 pairwise comparisons were significant for the various traits. Generally, for each character, allopatric conspecific populations clustered together to the exclusion of heterospecifics (Fig. 1). Nevertheless, exceptions to this pattern existed; pairs of heterospecific populations in many cases did not differ significantly. Further, no evidence of congruence occurred among characters, as the majority rule consensus of the five univariate dendrograms was completely unresolved.

Significant differences were detected between sympatric populations at three localities, involving three characters (Table 5). At both Black Rocks and Westwater Canyon, specimens of *G. robusta* had, on average, larger eyes and thicker caudal peduncles than did *G. cypha* individuals. In Westwater Canyon and Yampa River, the *G. cypha* sample showed greater concavity of the skull/anterodorsal hump than did *G. robusta*. In addition to the six significant comparisons, 11 other contrasts displayed expected trends between species; only 7 of 25 nonsignificant comparisons (three involving MA, three from Desolation Canyon species pairs) were inconsistent with general patterns.

Multivariate tests of differences among groups were highly significant (Wilks' lambda = 0.169, $F_{60, 1549} = 11.943$, $P < 0.001$). Four of five canonical vectors contained significant among-group structure. The first canonical vector (CV I) separated populations accord-

ing to species (with the exception of Desolation Canyon *G. robusta* and Cataract Canyon *G. cypha*). CV II contrasted Black Rocks, Westwater Canyon, and Yampa River populations of both species from all other populations. CV III–IV both displayed a complex pattern of scores that could not be readily interpreted independent of other axes.

Individuals from sympatric populations could be assigned to groups with a high degree of accuracy (Table 6); the pooled classification success rate exceeded 98%, and discrimination was >93% for all localities. Despite this level of sensitivity within localities, the discriminatory power across all populations was poor (39% correctly classified) and did not differ between species. Overall classification success rates did not exceed 60% for any of the 13 populations.

UPGMA clustering showed good separation of the two species (Fig. 2a); only the Desolation Canyon *G. robusta* population clustered with heterospecifics. The more conservative consensus topology (Fig. 2b) indicated the presence of five distinct clusters: (1) "allopatric" *G. cypha* from the Grand Canyon (where *G. robusta* no longer occurs); (2) "sympatric" *G. cypha* from localities (excluding Cataract Canyon) containing sympatric *G. robusta*; (3) Desolation Canyon fish of both species; (4) "allopatric" *G. robusta* from Debeque and Rifle (which lack *G. cypha*); and (5) "sympatric" *G. robusta* from localities that also harbor populations of *G. cypha*. This last cluster also contains the *G. cypha* population from Cataract Canyon.

Two characters (ED, SD) produced pairwise difference matrices that were significantly positively correlated ($r = 0.455$ and 0.608 , respectively; $P < 0.002$ for both) with a matrix of squared Mahalanobis distances among populations derived from a 56-character truss analysis (McElroy and Douglas 1995). The comparison involving OJ ($P = 0.02$) was marginally nonsignificant at Bonferroni-adjusted levels. The distance matrix resulting from multivariate analysis was also highly correlated with the truss-derived matrix ($r = 0.522$, $P < 0.002$). Despite these significant matrix correlations, however, consensus topologies based on the same pairs of matrices were poorly resolved.

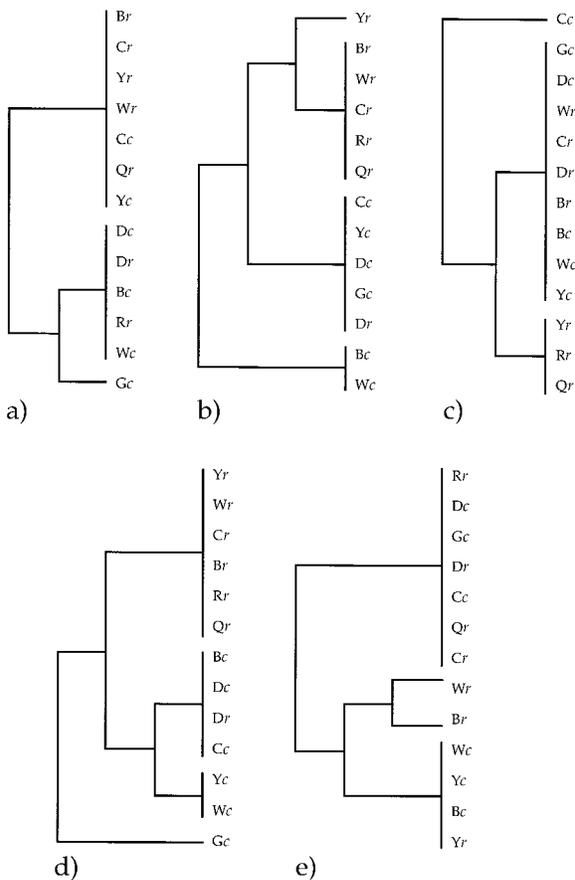


FIG. 1. UPGMA clustering of absolute pairwise differences among populations of *G. robusta* and *G. cypha* for each of five field characters. Dendrograms depict estimated hierarchical relationships based on (a) ED, (b) CP, (c) OJ, (d) SD, and (e) MA (see Table 1 for key to acronyms). Uppercase letters in population labels identify the locality (see Table 4) and lowercase letters indicate the species ($r = G. robusta$, $c = G. cypha$) comprising that population. For each plot, only those nodes at which some significant pairwise differences were detected in Tukey's HSD tests are shown as resolved; note that not all pairwise contrasts across a resolved node need be significant. Branch lengths are not indicative of the phenetic distances estimated among taxa. Operational taxonomic units are ordered by population means to facilitate comparison and oriented with putatively more "robusta-like" values at the top. For example, the Br population was characterized as having the largest mean eye diameter; as larger eyes have been associated with *G. robusta*, this population appears at the top of panel (a).

DISCUSSION

Significant differences exist at several levels both within and between the two *Gila* species examined. For individual characters and sites, sympatric species pairs can be readily differentiated. However, population means vary among localities, and individual fish often display a mosaic of traits. As such, field identification of a significant proportion of any specimens collected likely will be impossible. We consider the implications of these findings with respect to the population biology

and management of these unique fishes, use *Gila* as a case study highlighting problems with existing requirements in designating biological units for protection under the ESA, and suggest a means by which restoration priorities may be more appropriately and objectively evaluated.

Simple field characters can distinguish species

Significant differences in three univariate descriptors (ED, SP, CD) were consistent with previous contrasts of the two species (Miller 1946, Minckley 1973, Douglas et al. 1989). The pattern associated with OJ suggests that *G. cypha* has a smaller gape than does *G. robusta*; however, we interpret this finding cautiously, given that gape was measured indirectly. While OJ proved a useful discriminator, it is preferable to measure gape directly. In general, the univariate results suggest that a small number of composite characters may permit field workers to classify upper basin *Gila* with reasonable confidence across multiple localities.

Douglas et al. (1989) and Kaeding et al. (1990) both used a similar set of characters in principal component analyses of fish collected from the Yampa River and Black Rocks, respectively. In each case, characters analogous to CP and SD were important in defining clusters representing *G. robusta* and *G. cypha* individuals. Douglas et al. (1989) concluded that such qualitative characters might be particularly valuable for differentiating problematic taxa such as those comprising the *G. robusta* complex. Our above generalization is consistent with this view, but differs in two important respects. First, implementation of our approach allows unknown specimens collected in the future to be classified based on discrete criteria established herein (e.g., univariate sample means, discriminant function scores). Second, our data extend beyond a single locality to sites throughout the upper Colorado River basin, suggesting that these features are more generally applicable to species identification than could necessarily be inferred from samples collected at a single locality.

Nevertheless, it is also important to recognize that discriminatory power is not absolute and is to some extent locality specific. In particular, misclassifications are greatest at those sites in which hybridization may be occurring and the need for accurate identification may be most acute. Further, while 70–90% discrimination can be considered robust in a biological sense, the residual ambiguity (of whatever derivation) still compromises legal or technical designations of "species" currently required under the ESA.

Patterns of character variation are consistent, but congruence is limited

Despite considerable variability in population means among localities, sympatric species pairs generally display significant morphological separation. While we cannot say, for example, that a sample of *G. robusta* always has an eye diameter of $0.031 \times TL$ (the species

TABLE 5. Character trends among sympatric species pairs of *Gila robusta* and *G. cypha*. Values represent mean \pm 1 SE. Asterisks indicate comparisons that were significant ($P < 0.05$) based on Tukey's HSD tests for differences among populations. Mean \pm 1 SE for allopatric populations of *G. robusta* and *G. cypha* are also provided.

Locality	ED	CP	OJ	SD	MA
Black Rocks	*	*			
<i>G. robusta</i>	0.0332 \pm 0.0006	-0.0231 \pm 0.0022	0.0151 \pm 0.0010	0.0016 \pm 0.0011	41.732 \pm 1.083
<i>G. cypha</i>	0.0286 \pm 0.0006	0.0078 \pm 0.0037	0.0145 \pm 0.0009	0.0037 \pm 0.0010	38.884 \pm 0.805
Cataract Canyon					
<i>G. robusta</i>	0.0331 \pm 0.0008	-0.0174 \pm 0.0064	0.0154 \pm 0.0017	0.0015 \pm 0.0013	44.733 \pm 2.029
<i>G. cypha</i>	0.0314 \pm 0.0011	-0.0120 \pm 0.0038	0.0197 \pm 0.0020	0.0042 \pm 0.0021	45.491 \pm 2.184
Desolation Canyon					
<i>G. robusta</i>	0.0288 \pm 0.0007	-0.0004 \pm 0.0029	0.0153 \pm 0.0009	0.0039 \pm 0.0006	45.525 \pm 1.004
<i>G. cypha</i>	0.0294 \pm 0.0007	-0.0071 \pm 0.0041	0.0160 \pm 0.0011	0.0039 \pm 0.0009	47.618 \pm 1.158
Westwater Canyon	*	*		*	
<i>G. robusta</i>	0.0318 \pm 0.0004	-0.0219 \pm 0.0015	0.0154 \pm 0.0006	0.0004 \pm 0.0005	42.096 \pm 0.664
<i>G. cypha</i>	0.0275 \pm 0.0004	0.0138 \pm 0.0020	0.0140 \pm 0.0005	0.0071 \pm 0.0007	40.337 \pm 0.625
Yampa River				*	
<i>G. robusta</i>	0.0324 \pm 0.0004	-0.0311 \pm 0.0019	0.0115 \pm 0.0005	-0.0008 \pm 0.0004	38.832 \pm 0.593
<i>G. cypha</i>	0.0301 \pm 0.0015	-0.0106 \pm 0.0040	0.0133 \pm 0.0019	0.0056 \pm 0.0014	39.280 \pm 1.640
Debeque					
<i>G. robusta</i>	0.0305 \pm 0.0008	-0.0158 \pm 0.0036	0.0099 \pm 0.0009	0.0022 \pm 0.0008	45.225 \pm 1.250
Rifle					
<i>G. robusta</i>	0.0278 \pm 0.0007	-0.0170 \pm 0.0047	0.0107 \pm 0.0011	0.0020 \pm 0.0008	48.016 \pm 0.993
Grand Canyon					
<i>G. cypha</i>	0.0263 \pm 0.0006	-0.0020 \pm 0.0028	0.0162 \pm 0.0007	0.0093 \pm 0.0007	45.843 \pm 1.023

mean estimated from our sample), we can say that it is generally greater than that of *G. cypha* at a given locality. While raising interesting ecological questions regarding establishment and maintenance of this pattern in the face of potential genetic introgression (Dowling and DeMarais 1993, McElroy and Douglas 1995), this among-locality variability makes defining a classification criterion for general use problematical.

The lack of congruence in hierarchical phenetic relationships among populations as estimated by the various data sets is relevant to the search for field characters able to differentiate *Gila* species. Taking the topology derived from the prior truss analysis of these specimens (McElroy and Douglas 1995) as a baseline

of reality (given the degree of morphometric coverage of the specimens and sensitivity of the analysis), we can ask if any simple sets of features might serve as a proxy for that approach. Our results indicate that each field character suggests a different pattern of relationships, and none (nor their multivariate discriminant combination) approximates the truss-based topology. No single character appears to contain sufficient information to serve as a reliable marker of population distinctiveness throughout the upper Colorado Basin. While complementary genetic studies are in progress (T. Dowling, *personal communication*), we anticipate,

TABLE 6. Classification error rates between sympatric species pairs for the 13-group discriminant function.

Locality	Error rate
Black Rocks	0.000 (0/44)
Cataract Canyon	0.059 (1/17)
Desolation Canyon	0.069 (3/43)
Westwater Canyon	0.000 (0/110)
Yampa River	0.014 (1/70)
Overall	0.018 (5/284)

Note: Specimens used to compute the discriminant function were assigned to a species a posteriori based on their generalized squared Mahalanobis distance from the centroid of each group. Proportions indicate the number of individuals from a given locality misclassified as belonging to the population of the other species from the same locality. Values in parentheses represent the number of misclassifications/total number of individuals of both species from that locality classified.

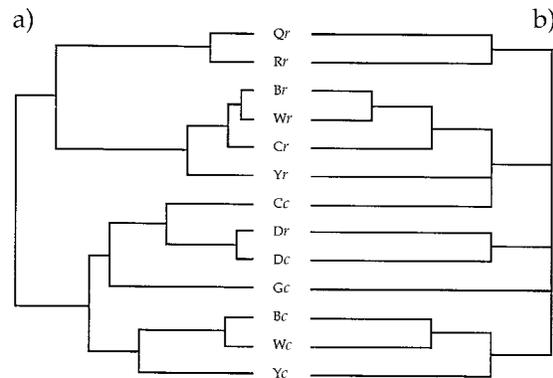


FIG. 2. Phenetic relationships among populations of *Gila robusta* and *G. cypha* as inferred by (a) UPGMA clustering and (b) strict consensus of single and complete linkage clustering of generalized squared Mahalanobis distances derived from canonical discriminant analysis of five morphological field characters. Population labels are as in Fig. 1.

based on existing data (DeMarais et al. 1992, Dowling and DeMarais, 1993), that genetic histories of these species will be similarly reticulate. From our perspective, continued emphasis on finding (or attempting to find) simple diagnostic features to delineate populations and species is unwarranted; rather, we should focus on developing coherent and biologically based management practices within the ecological and genetic parameters established by the organisms themselves.

Further evidence exists of a locality effect on phenetic relationships

McElroy and Douglas (1995) suggested that the morphological similarity of heterospecific populations at Cataract and Desolation canyons might reflect introgressive hybridization or convergent local adaptation at those sites. Cluster analysis based on the characters examined here suggests that this phenomenon may be more pervasive than originally proposed. The consensus of single and complete linkage dendrograms identified five discrete clusters, such that populations occurring sympatrically were distinct from conspecific populations existing in the absence of their congener. The distinctiveness of "sympatric" clusters in both species suggests that hybridization or local adaptation may be important biological and evolutionary forces at Black Rocks, Westwater Canyon, and Yampa River, in addition to Cataract and Desolation canyons. While visible in retrospect, this interpretation was not readily apparent from the earlier study (McElroy and Douglas 1995).

The "producer's vs. consumer's risk" gambit applies

Sets of characters do not provide substantially greater discrimination than do component features considered alone. An increase in classification efficiency provided by the discriminant function (7–14%) is due in part to differential weighting of variables by the estimated linear coefficients. When specimens are categorized based simply on the majority-rule consensus of classifications suggested individually by ED, CP, and SD, discriminatory power is not increased at all (overall percentage of correct assignments = 75.5%). In fact, the percentage of misclassifications is higher than would be predicted if character states assorted randomly (24.5% observed vs. 17.5% predicted under a binomial model). This suggests that individuals display a mosaic of traits characteristic of one or the other species. The largest coefficient of variation (r^2) computed for a pair of characters (CP and SD) pooled across species was 0.21. Because these key morphological features are relatively uncorrelated, the additive effect of multiple characters does not significantly increase classification efficiency.

By adjusting cutoff values for each character in a majority-rule classification scheme, it is possible to ac-

curately classify a higher proportion of specimens from one species or the other, but not both. As we correctly recognize more individuals of one species, decreasing the Type I error rate for that taxon, we simultaneously increase the Type II error rate. As a result, one group is defined inclusively, consisting of all individuals belonging to that group as well as some that do not, while the other becomes an exclusive category, containing only those specimens that can unambiguously be allocated to that group. This tradeoff between Type I and Type II errors is known as "producer's vs. consumer's risk," reflecting the differential willingness of these two groups to accept inferior quality merchandise (Sokal and Rohlf 1981).

We can use this dichotomy, as reflected by upper basin *Gila*, to consider relevant questions in conservation and management. Given that *G. cypha* appears to be in more immediate danger of extinction than *G. robusta*, and that at least the potential for introgressive hybridization exists at a number of localities where *G. cypha* is extant, how should we define and classify individuals putatively of this species? Is it more prudent to identify as *G. cypha* any fish that may contain *cypha* genes (the producer's risk), or is it better to restrict our envelope for this species to include only morphologically unambiguous (which may not equal "pure") representatives of this species (the consumer's risk)? Under the latter approach, we would likely underestimate *G. cypha* individuals in a sample, and might conclude that some isolated populations are already extinct. This could be particularly significant for Cataract Canyon, which harbors an unusually *robusta*-like form of *G. cypha*, but which has also been identified as one of three possible breeding populations remaining in nature (R. Valdez, *personal communication*). By contrast, a more inclusive definition would overestimate *G. cypha*, and might dilute the genetic integrity of the species through broad-brush conservation strategies. However, if hybridization between *G. robusta* and *G. cypha* represents an evolutionary strategy in this group (DeMarais et al. 1992, Dowling and DeMarais 1993), then genetic purity may be an irrelevant and perhaps detrimental goal (Dowling et al. 1992a, b, McElroy and Douglas 1995). Rather, we put forth the opinion that conservation goals for *Gila* may be best served by casting a wide management net and accepting the "producer's risk" strategy. In the present context, this would entail protecting the whole *G. robusta* complex (as defined in Douglas et al. 1989) under the ESA.

The gambit is a useful decision-making paradigm

Limited resources necessitate prioritization of recovery efforts for individual taxa (Carroll et al. 1996). In a recent assessment of ecological issues associated with the ESA, Carroll et al. (1996) argued that while existing priority systems based on magnitude and immediacy of threat, in addition to taxonomic distinct-

ness, are operationally advantageous (Tobin 1990), they would benefit from a broader ecological perspective. In particular, consideration should be given to the ecosystem importance and potential inclusive benefits arising from protection of a given endangered taxon (Carroll et al. 1996). Under these criteria, highest priority would be assigned to imminently threatened, ecologically important, and taxonomically distinct entities (Carroll et al. 1996).

We agree that more biological information should be brought to bear on decision-making processes; however, the issue of taxonomic distinctness introduces additional complexity that must be accommodated. This is particularly relevant with respect to the so-called "Hybrid Policy" (O'Brien and Mayr 1991, Avise 1994, O'Brien 1994), which traditionally devalued endangered taxa that displayed evidence of hybridization (USFWS 1996).

Numerous authors (O'Brien and Mayr 1991, Dowling et al. 1992a,b, Avise 1994, O'Brien 1994,) have legitimately criticized this across-the-board policy, though these and others have made different management recommendations for specific taxa. For example, O'Brien and Mayr (1991) argued for continued protection of the Florida panther as a distinct subspecies, suggesting that infusion of genes via introgression may actually have been beneficial to this genetically compromised and severely imperiled taxon. Similarly, in our view *Gila* warrants expanded intervention, as hybridization appears to increase the volume of the genetic reservoir shared by constituent species (Dowling and DeMarais 1993, McElroy and Douglas 1995). Dowling and Childs (1992) urged caution and advocated additional population genetic analyses prior to renovation of streams in which rainbow trout (*Oncorhynchus mykiss*) appear to have hybridized with native Apache trout (*O. apache*). Allendorf and Leary (1988), however, proposed eradication and replacement of cutthroat trout (*O. clarki*) populations with greater than 1% introgressed genes, citing concern that widespread hybridization could lead to homogenization of locally adapted populations and a decrease in fitness of lineages. These different perspectives indicate the need to evaluate ramifications of management decisions regarding hybrids (and taxonomic distinctness in general) on a case by case basis (Dowling et al. 1992a, b). This concept has since been adopted by the Fish and Wildlife and National Marine Fisheries Services in a proposed policy that would evaluate the potential impact (positive or negative) of at least some intercrosses and intercross progeny to the continued viability of an endangered parental taxon (USFWS 1996).

We suggest that the "producer's vs. consumer's risk" gambit represents a formalization of this view, and can thus aid in prioritizing objectives. As a statistical paradigm, it allows quantitative values of risk to be assigned to alternative decisions (Sokal and Rohlf 1981), given some knowledge of the frequency distribution of

character states in two populations (e.g., eye diameter, proportion of native vs. introduced alleles). At the same time, critical value of risk can be adjusted as a function of extenuating circumstances (such as the inclusive benefit or rarity of an endangered taxon). In this way, these ecological factors may be more appropriately balanced against the desire for taxonomic distinctness, both among individual taxa as well as temporally within the decline or recovery history of a single taxon. For example, the greater the danger of extinction of a taxon, the more inclusively it should be defined, such that the potential for preserving genetic variability and species viability is maximized (Echelle 1991). Here, the risk of extinction likely outweighs the risk or detriment of introgression, and this option would protect those species most in danger even though they may also be most likely to hybridize (Grant and Grant 1992). However, for a more abundant taxon, it may be preferable to minimize the risk of genetic homogenization by protecting more "pure" lineages. The approach we advocate reflects ongoing commonsense considerations, but is novel in that it accommodates the range of perspectives under a quantifiable statistical umbrella.

ACKNOWLEDGMENTS

We are grateful for the logistical support, cooperation, and personnel provided by the following agencies, organizations and/or institutions during the data collection phase of this project: Arizona Game and Fish Department (Phoenix); Arizona State University; Bio/West, Inc.; Brigham Young University; Bureau of Reclamation (Salt Lake City, Utah); Colorado Department of Wildlife (Denver; Grand Junction); National Museum of Natural History, U.S. Fish and Wildlife Service (Parker, Arizona; Grand Junction, Colorado. Vernal and Salt Lake City, Utah); and the Utah Division of Wildlife Resources (Moab; Price; Salt Lake City; Vernal). Scientific collecting permits were provided by the states of Arizona, Colorado, Utah, and from the U.S. National Park Service. An Endangered Species subpermit (PRT-676811) was provided by the USFWS. W. L. Minckley generously shared his perspectives on species identification under the ESA. John Lynch kindly served as a conduit for data being transferred between WKU and ASU. We appreciate the constructive comments made by Jamie Estill, Jeff Jack, Zack Murrell, and Michael Stokes during the drafting of this manuscript. This research was supported the Bureau of Reclamation under Contract I-CS-40-0970A to M. E. Douglas.

LITERATURE CITED

- Allendorf, F. W., and R. F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology* 2:170-184.
- Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York, New York, USA.
- Carroll, R., C. Augspurger, A. Dobson, J. Franklin, G. Orians, W. Reid, R. Tracy, D. Wilcove, and J. Wilson. 1996. Strengthening the use of science in achieving the goals of the Endangered Species Act: an assessment by the Ecological Society of America. *Ecological Applications* 6:1-11.
- 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 USA* 89:274-2751.
- Douglas, M. E. 1993. An analysis of sexual dimorphism in

- an endangered cyprinid fish (*Gila cypha* Miller) using video image technology. *Copeia* 1993:334–343.
- Douglas, M. E., and P. C. Marsh. 1996. Population estimates/population movements of *Gila cypha*, an endangered cyprinid fish in the Grand Canyon region of Arizona. *Copeia* 1996:15–28.
- 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.
- Dowling, T. E., and M. R. Childs. 1992. Impact of hybridization on a threatened trout of the southwestern United States. *Conservation Biology* 6:355–364.
- Dowling, T. E., and B. D. DeMarais. 1993. Evolutionary significance of introgressive hybridization in cyprinid fishes. *Nature* 362:444–446.
- Dowling, T. E., B. D. DeMarais, W. L. Minckley, M. E. Douglas, and P. C. Marsh. 1992a. Use of genetic characters in conservation biology. *Conservation Biology* 6:7–8.
- Dowling, T. E., W. L. Minckley, M. E. Douglas, P. C. Marsh, and B. D. DeMarais. 1992b. Response to Wayne, Nowak, and Phillips and Henry: use of molecular characters in conservation biology. *Conservation Biology* 6:600–603.
- Echelle, A. A. 1991. Conservation genetics and genic diversity in freshwater fishes of western North America. Pages 141–153 in W. L. Minckley and J. E. Deacon, editors. *Battle against extinction: native fish management in the American West*. University of Arizona Press, Tucson, Arizona, USA.
- Eisner, T., J. Lubchenco, E. O. Wilson, D. S. Wilcove, and M. J. Bean. 1995. Building a scientifically sound policy for protecting endangered species. *Science* 268:1231–1232.
- Franklin, J. F. 1993. Preserving biodiversity: species, ecosystems, or landscapes? *Ecological Applications* 3:202–205.
- Grant, P. R., and B. R. Grant. 1992. Hybridization of bird species. *Science* 256:193–197.
- 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. Schrader, 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.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27:209–220.
- McElroy, D. M., and M. E. Douglas. 1995. Patterns of morphological variation among endangered populations of *Gila robusta* and *Gila cypha* (Teleostei: Cyprinidae) in the upper Colorado River basin. *Copeia* 1995:636–649.
- Meacham, C. A. 1993. MorphoSys: an interactive machine vision program for acquisition of morphometric data. Pages 393–402 in R. Fortuner, editor. *Advances in computer methods for systematic biology*. Artificial intelligence, databases, computer vision. Johns Hopkins University Press, Baltimore, Maryland, USA.
- 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, W. L. 1973. *Fishes of Arizona*. Arizona Game and Fish Department, Phoenix, Arizona, USA.
- . 1991. Native fishes of the Grand Canyon region: an obituary. Pages 124–177 in *Colorado River ecology and dam management*. National Academy Press, Washington, D.C., USA.
- O’Brien, S. J. 1994. A role for molecular genetics in biological conservation. *Proceedings of the National Academy of Sciences (USA)* 91:5748–5755.
- O’Brien, S. J., and E. Mayr. 1991. Bureaucratic mischief: recognizing endangered species and subspecies. *Science* 251:1187–1188.
- Rising, J. D., and K. M. Somers. 1989. The measurement of overall body size in birds. *Auk* 106:666–674.
- Rohlf, F. J. 1993. NTSYS-pc, Version 1.80. Exeter Software, Setauket, New York, USA.
- 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. U.S. National Park Service Transactions Proceedings, Series 5:613–623.
- Sokal, R. R., and F. J. Rohlf. 1981. *Biometry*. Second edition. W. H. Freeman, New York, New York, USA.
- SYSTAT. 1992. SYSTAT for Windows: Statistics, Version 5 Edition. SYSTAT, Evanston, Illinois, USA.
- Tobin, R. 1990. *The expendable future: U.S. politics and protection of biological diversity*. Duke University Press, Durham, North Carolina, USA.
- U.S. Fish and Wildlife Service (USFWS). 1985. Endangered and threatened wildlife and plants: notices of completion of review for 1978 and of five-year review for species listed before 1976 and in 1979 and 1980. *Federal Register* 50:29900–29909.
- . 1987. Humpback chub, *Gila cypha*, recovery plan (review draft). U.S. Fish and Wildlife Service, Region 6, Denver, Colorado, USA.
- . 1994. Endangered and threatened wildlife and plants; animal candidate review for listing as endangered or threatened species; proposed rule. *Federal Register* 50:CRF-part 17.
- . 1996. Endangered and threatened wildlife and plants; proposed policy and proposed rule on the treatment of intercrosses and intercross progeny (the issue of “hybridization”); request for public comment. *Federal Register* 61:4710–4713.
- Wayne, R. K. 1992. On inferring hybridity from morphological intermediacy. *Taxon* 41:11–23.
- Wayne, R. K., and S. M. Jenks. 1991. Mitochondrial DNA analysis supports extensive hybridization of the endangered red wolf (*Canis rufus*). *Nature* 351:565–568.