



Analysis of Sexual Dimorphism in an Endangered Cyprinid Fish (*Gila cypha* Miller) Using Video Image Technology

Author(s): Michael E. Douglas

Source: *Copeia*, Vol. 1993, No. 2 (May 3, 1993), pp. 334-343

Published by: American Society of Ichthyologists and Herpetologists

Stable URL: <http://www.jstor.org/stable/1447134>

Accessed: 11/01/2010 20:13

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/page/info/about/policies/terms.jsp>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/action/showPublisher?publisherCode=asih>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.



American Society of Ichthyologists and Herpetologists is collaborating with JSTOR to digitize, preserve and extend access to *Copeia*.

<http://www.jstor.org>

Analysis of Sexual Dimorphism in an Endangered Cyprinid Fish (*Gila cypha* Miller) Using Video Image Technology

MICHAEL E. DOUGLAS

Univariate and multivariate techniques were used to evaluate sexual dimorphism in 53 morphometric measures taken from 63 adult specimens of an endangered cyprinid, the humpback chub (*Gila cypha*). Specimens were filmed and released unharmed after their capture in the Colorado and Little Colorado rivers of the Grand Canyon (Arizona). Morphometric data were later extracted from film using a microcomputer and image analysis software. Because of the unique morphology of this fish, analyses emphasized its anterodorsal hump. Only two of 53 characters (3.8%) revealed significant sexual dimorphism in an analysis of covariance; approximately what one would expect from chance alone (i.e., one in 20, or 5%). A discriminant analysis correctly classified specimens by sex only 60% of the time, which is not significantly different from random expectation. Multiple group principal component analysis (MGPCA) and sheared PCA using all 53 characters also failed to delineate significant sexual dimorphism. Morphological shape differences among individuals, regardless of sex, were clearly apparent from character loadings onto both principal component 2 and sheared principal component 2. These differences were due to extent and development of the nuchal hump and to concomitant changes in concavity and length of the head which accompany its development. These differences appear ontogenetically based and are unrelated to either sexual or seasonal variation.

NATIVE fishes inhabiting large rivers of western North America are unique, because their phenotypes have been molded over evolutionary time by the unusually harsh conditions of a turbulent and sediment-rich habitat (Miller, 1961; see Carlson and Muth, 1989, for a description of the mainstream Colorado River). As a result, many of these fishes evolved a characteristic morphology: scalloped crania, humped or crested dorsums, thin but rigid caudal peduncles, and large, falcate fins. These characteristics are believed to have evolved in response to intense selective pressure for stability and maneuverability in swift currents and for explosive propulsion through turbulent, boulder-strewn rapids. A reduction in relative eye size, coupled with an imbedding of scales into epidermis, are suspected to be adaptive responses to heavy (preimpoundment) sediment loads in the rivers (Miller, 1946; Minckley, 1973). These fishes frequently attain relatively large body sizes (to 40 cm TL, or more) and are either known, or suspected, to achieve considerable longevity (Rinne et al., 1986; McCarthy and Minckley, 1987). Adult size and longevity both suggest an adaptive strategy (Wylbur et al., 1974) evolved in response to the tremendous environmental variability inherent in preimpoundment western rivers, such that the species maintains itself in spite of successive failed breeding seasons or lost year-classes. The

majority of these fishes are now listed as endangered (or are candidates for such listing; see Minckley and Douglas, 1991) due to numerous recent modifications of the riverine habitat by modern humans.

The humpback chub (*Gila cypha*, Family Cyprinidae; Fig. 1B), described from Bright Angel Creek (Miller, 1946) in Grand Canyon National Park (GCNP), is one of the most morphologically bizarre fishes on this continent (Rolston, 1991:94). According to Miller (1946:415), "*Gila cypha* . . . judged from its large, falcate fins, specialized nuchal hump, inferior mouth, and dorso-ventrally flattened head . . . is well adapted for life in the swift current very near or on the bottom. The action of the current against the prominent nape tends to force the fish down towards the bottom or the sides, where the flow is not so torrential as in mid-water. The small eye may represent a degeneration correlated with reduced light due to excessive silt, or it may be a response to the direct effect of the scouring action of the suspended matter, or both." Minckley (1973:98-99) elaborated further, stating that ". . . the belly and lower part of the head are flattened, the mouth is essentially horizontal and overhung by a produced, fleshy snout. It would seem that the hump acts as a barrier to passing water, forcing the fish's body against the bottom. Perhaps the pronounced grooves laterally on the hump, and

leading ventro-posteriorly directly to the upper part of the gill openings, allow water to irrigate the gills when the mouth and body are pressed downward. The ventral mouth would therefore be usable, when protected from the onrushing water by the snout (in a manner similar to that occurring in some swift-water suckers)."

In spite of Miller's (1946) diagnosis, the taxonomic validity of *G. cypha* has been questioned, most often by personnel from state and federal agencies who monitored and assessed riverine populations of *Gila* (Holden, 1991). In the 1950s and 1960s, these individuals commonly recognized only two subspecies of chub in the Colorado River: Roundtail (*G. r. robusta*) and bonytail (*G. r. elegans*). The humpback chub was simply considered a male bonytail, with the nuchal hump a secondary sexual characteristic (reviewed by Holden, 1991:49). Several studies (e.g., Holden and Stalnaker, 1970; Suttkus and Clemmer, 1977; Smith et al., 1979) have since recognized the three Colorado River chubs as distinct species (see Douglas et al., 1989 for a review of the nomenclatorial history of the *G. robusta* complex). The nuchal hump as a sexual characteristic has yet to be addressed.

Gila cypha is part of an endemic Colorado River Basin fauna that extends at least as far back as the Miocene (Miller, 1959; Minckley et al., 1986). It has been recorded from deposits dated to 4000 B.C. in the Grand Canyon (Miller and Smith, 1984) and from Native American ruins near Hoover (Boulder) Dam (Miller, 1955). There are only a few published studies on the ecology of *G. cypha* (Kaeding and Zimmerman, 1983; Kaeding et al., 1990), and knowledge about its morphological variation is primarily comparative (i.e., relative to other members of the *G. robusta* complex: Douglas et al., 1989; Kaeding et al., 1990; R. Valdez, Bureau of Reclamation, 1990, unpubl., and references therein). Suttkus and Clemmer (1977) evaluated morphological variation within *G. cypha*, but their results were limited by small samples for various size-groups and by modest statistical analyses.

There are reasons for the paucity of published data on *G. cypha*. Most research on this species has been government sponsored (accomplished either directly by agency employees or via contracts from agencies to independent researchers). Results of these studies are produced as "technical reports" or other agency-sponsored (i.e., "gray") literature, which are published without adequate review and without broad distribution (Collette, 1990). These reports do have a function in that they provide the agency with a record of the type of research

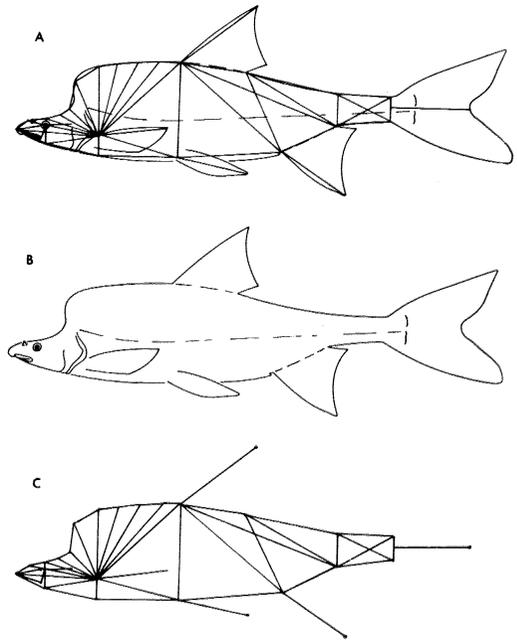


Fig. 1. (A): Fifty-three-character truss superimposed upon the phenotype of an adult *Gila cypha*; (B): An adult *Gila cypha* without truss; (C): Fifty-three-character truss framework alone.

performed and the results obtained. Yet, agency reports are often cited as if they are readily available, when in fact they often are not. Many journals (such as *Copeia*) will not, in fact, accept as valid literature citations technical reports published by the majority of state and federal agencies. To circumvent this problem, agencies should either avoid (Collette, 1990), or at least minimize (Wilbur, 1990), production of gray literature and instead emphasize publishing of technical results in the formal literature.

Limited published data on *G. cypha* also reflects the biology of the species itself. For example, humpback chub inhabit narrow, canyon-bound segments of the Colorado River and its major tributaries (Minckley, 1973; Holden and Minckley, 1980; Tyus et al., 1982). Access to these sites is difficult or impossible; research on the species is thus logistically difficult and expensive. In addition, the species has only been recognized for less than 50 years; as a result the literature base is quite modest, and museum specimens suitable for morphological study are scarce. Field studies to collect additional specimens are further constrained by its endangered status.

The present study was undertaken with two goals in mind: (1) to quantitatively evaluate morphological variation in this species, with particular emphasis on sexual dimorphism as it

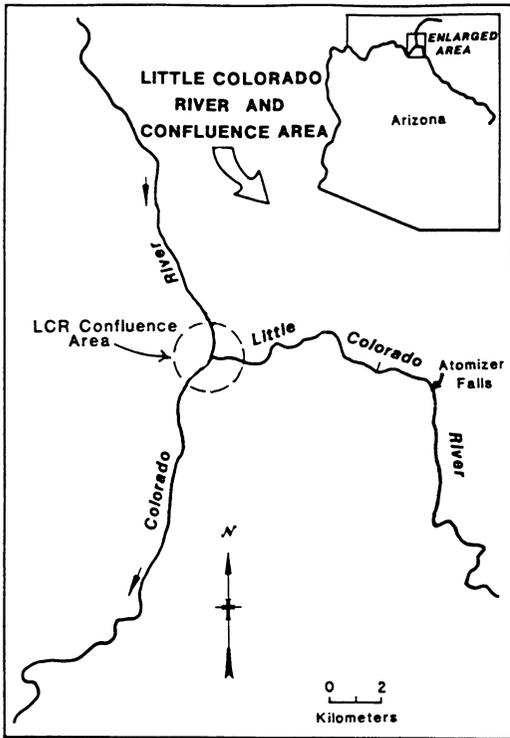


Fig. 2. Map depicting the Little Colorado River (Navajo Indian Reservation, Coconino County, Arizona), at its confluence with the Colorado River (Grand Canyon National Park, Coconino County, Arizona).

relates to the unusual anterodorsal (nuchal) hump (Fig. 1B); and (2) to perform this evaluation using image-analysis technology, which permits rare and/or endangered fish to be captured, filmed, and released unharmed. The hypothesis under test in this report is that sexual dimorphism is morphologically pervasive in *G. cypha*, with the distinct nuchal hump of this species its primary focus.

METHODS AND MATERIALS

Collection of video images.—*Gila cypha* were collected with hoop and trammel nets by Arizona Game and Fish Department (AZGF) during two time periods: in May 1988 at the confluence of the Colorado and Little Colorado rivers (GCNP), and in May 1989 at the confluence and in the Little Colorado River 11 km upstream from the confluence (Navajo Indian Reservation; see Fig. 2). Adult humpback chub (i.e., those greater than 230 mm TL) were immersed in a 19-liter bucket of river water treated with MS-222 (Sandoz Laboratories). Comatose fish were either Carlin or PIT tagged and sexed using external

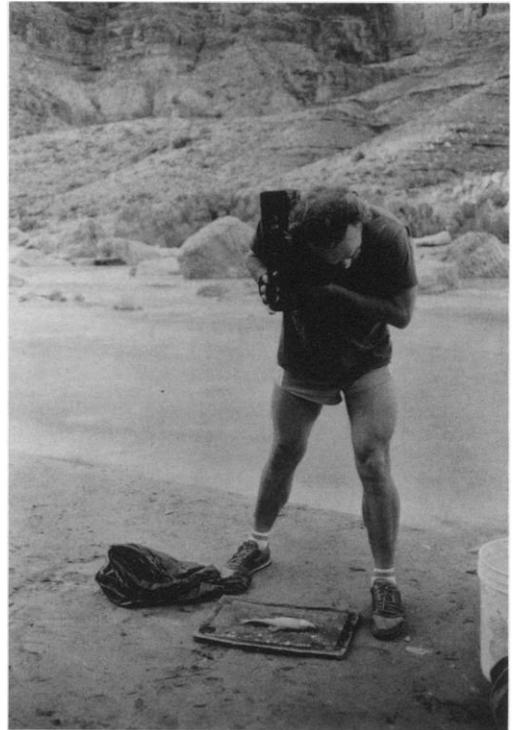


Fig. 3. Use of camcorder to film *Gila cypha* along shore of the Little Colorado River (Navajo Indian Reservation, Coconino County, Arizona).

morphology of the urogenital papillus (Suttkus and Clemmer, 1977:4; B. L. Jensen, USFWS, pers. comm.). Chubs were then rewetted and placed onto a styrofoam board (50 × 30 cm; Fig. 3) that had been previously spray painted a neutral color and indented with a shallow depression for the body of the fish. A 10-cm rule was placed beside the fish to provide scale. Dorsal and anal fins were quickly spread and pinned using insect pins, and the chub was filmed for approximately 10 sec (Fig. 3), using a 16mm camcorder (General Electric model 9-9808 SE with a 7-lux low light capability and an automatic focus 6:1 zoom lens). Fish were then returned to a 19-liter bucket of fresh river water, maintained there until they revived (i.e., were able to right themselves and respond to stimuli), and were then released back into the river.

Capture of video images.—Video images were copied from tape and analyzed at Arizona State University (ASU), Tempe. The image analysis system consisted of a Zenith 12 mhz 80286 microprocessor with Intel 80287 math coprocessor, 80-megabyte hard drive, and Mountain Filesafe TD-4000 (40 megabyte) tape backup. A NEC Multi-Sync monitor was used for image

display, while a Zenith 1490 flat-screen monitor displayed programming commands. The NEC was harnessed to a Matrox PIP 640-B frame-grabber board fitted into the microcomputer for image capture. Software to drive the system (i.e., to store, retrieve, and manipulate images and to extract and save morphometric measurements from these images) was produced by Bio-Scan, Inc. (Everett, Washington) and marketed under the name "Bio-Scan Optimas." The software operated within the Microsoft Windows environment and was mouse driven.

Eighty adult *G. cypha* were filmed during 1988 and 1989. Images were evaluated by first wiring the camcorder to the frame-grabber board of the microcomputer and then operating the camcorder in VCR mode. When a focused image was displayed on the monitor, the camcorder/VCR was halted and the image saved onto hard disk using the Optimas software. Each recorded image was thus a single "frame" selected from the 10-sec series of frames recorded in the field.

Extraction of morphological data.—Of the 80 taped images, 63 (29 male, 34 female) were of suitable resolution for detailed morphological analysis. In the first step, the 10-cm rule in the image was digitized (in mm) to serve as a measurement standard for that image. A truss (Strauss and Bookstein, 1982) consisting of 53 individual measures was then superimposed onto the image (Fig. 1A). During this process, special attention was afforded the head and nuchal hump (Fig. 1C). Anatomical landmarks (Strauss and Bookstein, 1982) from which to position the truss were scant in the nuchal region. To compensate, a series of computed "landmarks" were produced. Eight of these were located on the dorsal edge of each image from snout to origin of the dorsal fin in the following manner. First, three anatomical landmarks (i.e., pupil of eye, nape of head, and origin of pectoral fin) were identified. The horizontal coordinates of these three landmarks (produced by the software) were projected vertically to the dorsal edge of the image and recorded. Then, four additional points were positioned along the snout-to-dorsal origin to be equidistant between each of the three projected points and the endpoints. For example, a point equidistant between snout and vertical-from-pupil was produced by subtracting the x-coordinate of the former from that of the latter and dividing by two. The distance between the last two points in this series (i.e., between vertical-from-pectoral and origin-of-dorsal) was considerably larger than the preceding distances. Because this region was

crucial for demarcating the nuchal hump, two additional points were located between these landmarks, effectively dividing this last segment into quarters. Consequently, 11 total landmarks (three bona fide and eight calculated) were positioned along this anterior dorsal margin: Characters (9)–(19) (Table 1; Fig. 1A, 1C) represent distances connecting each of these 11 points to the pectoral origin. Characters (20)–(29) connected the same 11 points to one another in a linear sequence from anterior (i.e., snout) to posterior (i.e., dorsal origin). The other 32 truss measures outlined the remainder of the image and summarized various aspects of head, body, and peduncle, plus fin lengths (Table 1; Fig. 1A, 1C).

The resulting matrix of 63 fish by 53 distances was migrated to the ASU IBM-3090-5000 mainframe supercomputer for analysis using the Numerical Taxonomy System of Multivariate Statistical Programs (NT-SYS; F. J. Rohlf, J. Kishpaugh, and D. Kirk, 1974, unpubl. tech. rept., State University of New York, Stony Brook), the Statistical Analysis System (SAS, 1985), and BioMedical Statistical Software (BMDP; Dixon, 1990).

Data analysis.—All data were first transformed to base-10 logarithms, then grouped by sex and tested for skewness and kurtosis. The variance/covariance matrices for both sexes were then tested for equality. Correlations were calculated amongst characters over all individuals, regardless of sex. Arithmetic means and standard deviations were then calculated by sex for each character. To test each variable for sex-related differences, an analysis of covariance (ANCOVA) was performed, with the longest linear distance (i.e., that between pectoral and pelvic fins) serving as the covariate. This approach removed variation in a particular measurement correlated with overall body size. Mean measurements for each sex were subsequently adjusted to a common body size, allowing a more equitable comparison of measurements on the two sexes.

Variables were then standardized on the basis of pooled, within-sex standard deviations (Rohwer and Kilgore, 1973; Schnell et al., 1985). Standardization was accomplished by subtracting the value of a given character from the grand mean of that character averaged over both sexes. This value was then divided by the pooled, within-group standard deviation of the character (see Rohwer and Kilgore, 1973:160, fig. 2). This technique has advantages over other standardization methods because more emphasis is given to those characters possessing a rel-

TABLE 1. CHARACTER NAMES, ARITHMETIC MEANS, STANDARD DEVIATIONS, PRINCIPAL COMPONENT (PC) LOADINGS, SHEARED (=H2) PC LOADINGS, AND ANCOVA F-RATIOS FOR MALE AND FEMALE *Gila cypha*.

Character number/name*	Mean		SD		PCI	PCII	H2	F-ratio
	M	F	M	F				
1 Sn-Mouth corner	20.2	20.3	3.9	4.8	0.85	-0.13	0.08	2.09
2 Mouth corner-Pu	11.2	11.2	2.4	2.1	0.79	0.07	-0.04	2.18
3 Pu-VPu	5.4	6.2	1.3	1.9	0.56	-0.39	0.26	1.11
4 Pu-DPu	15.8	15.6	3.3	2.9	0.73	0.28	-0.20	1.91
5 OPe-DPe	11.7	12.2	3.1	2.8	0.72	0.22	-0.15	0.03
6 Sn-AnEye	17.8	17.4	3.6	3.3	0.82	-0.13	0.09	3.11
7 AnEye-PEye	8.4	8.6	1.3	1.9	0.72	0.08	-0.05	0.54
8 PEye-UpOp	24.8	27.1	4.7	5.4	0.93	0.05	-0.04	0.95
9 OPe-Sn	58.2	59.3	10.1	10.2	0.95	-0.24	0.16	1.06
10 OPe-VPu	39.6	40.9	7.2	7.1	0.92	-0.32	0.21	0.16
11 OPe-1/2(Sn-VPu)	48.7	50.0	8.6	8.6	0.94	-0.28	0.19	0.59
12 OPe-Na	31.6	31.8	5.8	5.8	0.86	-0.37	0.25	1.18
13 OPe-1/2(VPu-Na)	34.2	35.0	6.1	5.8	0.88	-0.40	0.26	0.42
14 OPe-Vpe	35.1	37.6	6.8	8.1	0.96	-0.17	0.11	0.03
15 OPe-1/2(VPe-Na)	33.6	35.8	6.8	7.7	0.94	-0.23	0.15	0.02
16 OPe-ODo	78.0	84.7	13.3	16.8	0.99	0.14	-0.10	1.08
17 OPe-1/2(VPe-ODo)	50.9	55.6	8.5	11.6	0.99	0.05	-0.03	0.89
18 OPe-1/2(1/2 ODo-VPe, VPe)	41.2	44.9	7.3	9.5	0.99	-0.07	0.05	0.50
19 OPe-1/2(ODo, 1/2 ODo-VPe)	63.7	69.5	10.7	14.0	0.99	0.10	-0.07	1.25
20 Sn-(1/2 S-VPu)	11.1	10.9	2.0	1.8	0.84	-0.12	0.08	3.38
21 (1/2 S-VPu)-VPu	10.8	10.6	2.1	1.8	0.84	-0.06	0.04	3.80
22 VPu-(1/2 VPu-Na)	8.6	9.8	1.8	2.2	0.86	0.21	-0.14	3.30
23 (1/2 VPu-Na)-Na	9.1	10.2	2.2	2.5	0.85	0.18	-0.12	1.30
24 Na-(1/2 Na-VPe)	10.0	11.1	2.9	3.8	0.71	-0.49	0.32	0.16
25 (1/2 Na-VPe)-VPe	8.6	9.0	2.0	2.8	0.58	-0.64	0.42	0.11
26 VPe-(1/4 VPe-ODo)	16.9	18.3	3.1	3.9	0.91	0.28	-0.19	0.33
27 (1/4 VPe-ODo)-(1/2 VPe-ODo)	17.0	18.0	3.3	3.8	0.88	0.31	-0.21	0.00
28 (1/2 VPe-ODo)-(3/4 VPe-ODo)	17.0	18.2	3.4	3.5	0.93	0.15	-0.10	0.26
29 (3/4 VPe-ODo)-ODo	17.3	18.2	3.7	3.5	0.91	0.19	-0.13	0.02
30 ODo-IDo	39.1	41.4	7.4	7.6	0.91	0.04	-0.03	0.10
31 OA-IA	37.7	40.3	6.8	7.6	0.95	0.09	-0.06	0.37
32 IA-VIA	23.6	26.1	4.4	5.5	0.98	0.13	-0.09	1.72
33 VenPd-DorPd	14.1	16.1	3.0	3.9	0.94	0.17	-0.14	2.85
34 IA-DorPd	50.4	56.7	10.2	13.1	0.96	0.12	-0.08	1.92
35 VIA-VPd	52.2	56.1	11.2	11.9	0.95	0.11	-0.07	0.10
36 AntDor-IA	85.1	88.5	13.1	14.4	0.98	0.01	-0.01	0.35
37 OA-IDo	47.1	51.7	8.1	10.6	0.99	0.13	-0.09	1.53
38 ODo-OA	68.9	72.8	11.5	13.1	0.98	0.05	-0.03	0.01
39 IDo-IA	48.0	50.2	7.9	8.5	0.93	0.00	-0.00	0.01
40 OA-OP1	52.5	56.1	9.2	11.3	0.92	0.11	-0.08	0.25
41 OP1-ODo	59.7	64.0	11.6	12.0	0.97	0.10	-0.07	0.27
42 IDo-VIA	36.3	37.2	6.7	6.6	0.78	-0.06	0.04	0.30
43 VIA-DorPd	48.4	52.4	10.4	11.5	0.94	0.10	-0.06	0.22
44 VenPd-IA	47.8	52.7	10.1	11.8	0.95	0.11	-0.07	0.88
45 OP1-DPe	54.5	56.2	8.5	10.5	0.94	0.17	-0.11	0.81
46 OP1-OPe	57.7	59.2	9.4	10.9	0.93	0.21	-0.14	1.20
47 DPe-DPu	35.1	37.0	6.7	6.8	0.93	-0.28	0.19	0.01
48 DPu-Sn	29.3	28.9	5.5	5.1	0.91	-0.01	0.01	4.40*
49 Ln Pe	47.1	49.6	9.7	11.5	0.92	0.08	-0.05	0.50
50 Ln Pl	36.8	40.0	7.6	9.1	0.94	0.00	-0.00	0.04
51 Ln Do	44.2	47.6	8.6	8.7	0.88	-0.04	0.03	0.48
52 Ln A	36.2	35.8	7.0	7.2	0.78	-0.06	0.04	2.02
53 Ln (DorPd-VenPd)-Fk	40.6	39.0	7.6	6.3	0.75	-0.04	0.03	4.40*

* Abbreviations as follows: A = Anal Fin; An = Anterior; D = Descend; Do = Dorsal Fin; Dor = Dorsal; Fk = Fork; I = Insertion; Ln = Length; Na = Nape; O = Origin; P = Posterior; Pd = Peduncle; Pe = Pectoral Fin; Pl = Pelvic Fin; Pu = Pupil; Sn = Snout; Up = Upper; Ven = Ventral.
* P < 0.05.

atively low within-group to total variance ratio (i.e., the potentially more discriminating characters). The variance-covariance matrix of standardized variables was analyzed using stepwise multiple discriminant analysis (SMDA program 7M of BMDP; Dixon, 1990), to identify those morphological variables (if any) that best distinguished the two sexes. Males and females were allocated to group using a jackknifed classification function (where individuals were successively left out of the derivation of the classification function and used later to test the function's efficacy). Jackknifed group totals by sex were then tested against chance-corrected expectations using Cohen's kappa statistics (Titus et al., 1984). A histogram of discriminant scores was plotted to illustrate the magnitude of sexual dimorphism (if any).

Standardized data were evaluated using multiple group principal component analysis (MGPCA) of NT-SYS. Scores for individuals were plotted in component space to illustrate this multivariate summary, and loadings for each character (i.e., correlations of characters with components) were tabulated.

PCA [as conceived by Hotelling (1933)] is a computational methodology applicable to but a single group. If a given study involves multiple groups (as in Jolicoeur and Mosimann, 1960; this, and probably most studies), the simplest approach is to generate individual PCAs for each group (as in Rising and Somers, 1989). However, in most circumstances, researchers tend to ignore group structure and instead apply ordinary PCA to data pooled over all groups (reviewed by Airoidi and Flurry, 1988). But pooling of data in this manner is clearly inappropriate. Directionality of a given component (i.e., its eigenvector) is determined by both between- and within-group variability, and pooling over all groups inextricably mingles both, thus confounding the component. Thorpe (1983) instead advocated derivation of principal components after pooling the variance-covariance matrices of all groups (a procedure called multiple group PCA; MGPCA). This approach has its advantages, in that variation between groups is not confounded by that found within groups. However, before variance-covariance matrices can be pooled, eigenvectors must be identical within groups. If this is not so, the group with the greatest variability will often determine the directionality of extracted components (i.e., dominate the eigenvectors). Thus, when using MGPCA, it becomes imperative to test equality of within-group covariance matrices (i.e., their eigenvalues and eigenvectors), as was done in this study. This has led some researchers (i.e.,

Airoidi and Flurry, 1988) to argue that MGPCA is less applicable in most circumstances, because of its more stringent requirements (but see Thorpe, 1988).

Sheared PCA (Bookstein et al., 1985; with corrections outlined in Rohlf and Bookstein, 1987) was also employed to evaluate size-free body-shape relationships amongst individuals from each sex. The sheared PCA was calculated from standardized data using PROC MATRIX (SAS, 1985; modified from an algorithm written by L. Marcus).

RESULTS

Univariate analyses.—All transformed variables were normally distributed, and the variance-covariance matrices for both sexes did not differ significantly ($P > 0.10$; Chi-square test of homogeneity of within-group covariance matrices, Proc Discrim; SAS, 1985). Correlations among the 53 pooled characters were positive, ranging from 0.031–0.995. No statistical differences were found between the slopes of the regressions for each sex (ANCOVA of Proc GLM; SAS, 1985). Only two characters [(48): tip of snout to descent of pupil, and (53): peduncle length] exhibited significantly different adjusted means (or intercepts) between males and females [(48): $F = 4.40$, $P < 0.04$; (53): $F = 4.40$, $P < 0.04$]. Table 1 lists all 53 variables, with their untransformed means, standard deviations, and F-values for the ANCOVA.

Discriminant analysis.—The discriminant function separating males and females incorporated three characters [i.e., (22): distance from vertical pupil to midpoint between vertical pupil and nape; (33): distance from anterior insertion of dorsal to anterior insertion of anal; and (48): distance from snout to vertical descent from pupil]. This function (not presented) was based on equal probability of a particular specimen being male or female. A jackknifed classification procedure demonstrated that only 58.6% of the males (17/29) and 61.8% of the females (21/33) could be correctly classified to sex. These group totals are not significantly different from chance alone (Kappa = 0.203, $Z = 1.61$, $P > 0.11$). The average number of correct classifications for both sexes was only 60.3%, slightly greater than half. A histogram of canonical scores for individuals of both sexes is provided in Figure. 4.

Principal component analysis.—The PCA revealed general trends in morphological variation over the 53 characters. The first four com-

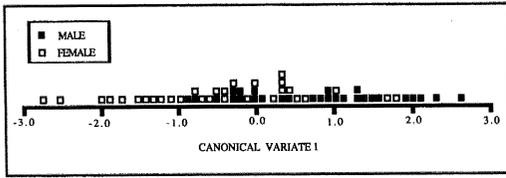


Fig. 4. Projections of 29 male (solid bars) and 34 female (open bars) humpback chubs onto the first discriminant function axis designed to separate the sexes. Some individuals are superimposed in drawing.

ponents explained 87.1% of the observed morphological variation.

The first component (PC1), which explained 78.1% of the total variation, provided no separation between males and females (Fig. 5). This component had high correlations with essentially all characters (Table 1), suggesting that it represents a general body size factor. The distribution of males and females overlapped considerably along this component (Fig. 5).

The second component (PC2) explained considerably less of the total variation (i.e., 4.2%; eigenvalue = 2.24), with only 11 relatively important characters (i.e., with loadings > 0.25). These characters are the vertical distances from pupil to dorsal (3) and ventral (4) aspects of the head; distances from pectoral origin to various landmarks on the dorsal edge of the head (i.e., 9–13); and the distances between discrete landmarks along the nuchal hump and those immediately preceding it (i.e., 24–27; see above). Thus, PC2 was interpreted as a head depression-head length-nuchal hump component. However, no separation of males from females was apparent on this axis (Fig. 5).

The third and fourth components explained 2.9% and 2.5% of the variability, respectively. These components summarized a modest amount of the total morphological variation and appeared to be biologically uninterpretable. Plots of PC3 and PC4 against one another, or against the other components (not shown), did not segregate males from females.

Sheared principal component analysis.—Three size-free components (i.e., H2, H3, H4) were generated using sheared PCA, but only loadings for the first (which accounted for 92% of the size-free variation) are presented in Table 1. Those variables which loaded heavily onto H2 were the same variables that loaded heavily onto PC2 [i.e., characters (3), (4), (10)–(13), and (24)–(27); character (9) was not important in H2]. Loadings for all characters were slightly lower than those recorded in the initial PCA (reduc-

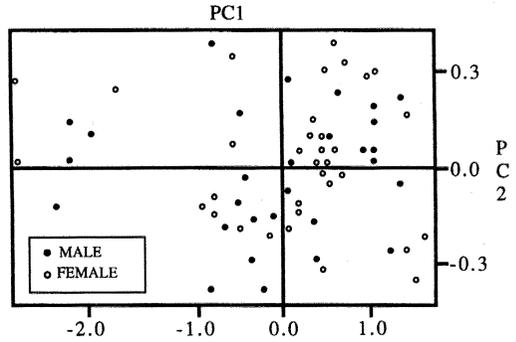


Fig. 5. Humpback chubs plotted with respect to the first two principal components, based upon 53 measurements. Characters were standardized on the basis of within-sex standard deviations.

tions ranged from 0.08–0.22, Table 1). These reductions suggest that, although body size contributed to most of the morphological variation, size-free shape variation was also present. As before, males and females were completely intermingled on the sheared PC axes (much as in Fig. 5). Plots of sheared components are, thus, not presented.

DISCUSSION

In this study of morphological variation in *G. cypha*, the influences of geographic and temporal variation were minimized by collecting specimens within a short time span and from a relatively small geographic area (as in Schnell et al., 1985). Variation associated with ontogeny (i.e., growth), however, is pervasive in these (and most) western North American fishes and cannot be adequately dealt with unless individuals are in some way aged. Because of the nondestructive sampling approach used, specimens were not aged in this study. However, by selecting adult-sized fishes (greater than 230 mm TL), ontogenetic variation was at least reduced in significance.

Intercorrelations between many of the characters were relatively low, suggesting that, although some individuals were larger (or smaller) than others (e.g., see PC1; Fig. 5), there were proportional and shape differences among individuals rather than between sexes. Two morphological characters differed statistically between sexes (ANCOVA, Table 1); however the magnitudes of these differences were, from a statistical standpoint, only marginal. In fact, given the large number of characters evaluated, one out of 20 (i.e., 5%) should be significant because of chance alone. Hence, finding two

modestly different characters out of 53 (i.e., 3.8%) is certainly not evidence for pronounced sexual dimorphism. If, in fact, a conservative Bonferroni approach (Harris, 1975:96–101) is used to compensate for the evaluation of multiple tests, the probability value for acceptance of a significant ANCOVA would be $P > 0.001$ (0.05/53). Hence, no characters would be judged significant in the ANCOVA.

Besides, one could argue that sexual dimorphism is an evolutionary phenomenon that is generally pervasive when it occurs, being exhibited in numerous phenotypic characters (see for example, Schnell et al. 1985; Douglas et al., 1986). The two allegedly dimorphic characters in this analysis displayed little apparent function in sexual display or sexual attraction among cyprinids, which further challenges the idea that variation in these characters was sexually based.

Results of the discriminant analysis (Fig. 4) further discourage the idea that sexual dimorphism is pervasive in *G. cypha*. Whereas the discriminant function was composed of three characters [i.e., characters (22), (33), and (48); Table 1], only 60% of all individuals could be correctly segregated to sex through its application. This was scant improvement over the original hypothesis that group membership for a given individual was of equal (e.g., 50%) probability.

Morphological shape differences among individuals in this study are apparent, regardless of sex. These differences are primarily due to extent and development of the nuchal hump and to concomitant changes in concavity and length of the head, which accompanies this development (PC2 and H2, Table 1). For example, distance from pupil to top of head [character (3)] decreases as distance from pupil to bottom of head [character (4)] increases. These modifications point to the development of the scalloped cranium characteristic of mature *G. cypha* (Fig. 1B). In addition, as scalloping proceeds, there is a reduction in four distance measures that extend from pectoral origin to various landmarks along the dorsal edge of the head and nape [characters (9)–(12), Table 1]. These reductions suggest a tendency for the head (from snout through approximately the nape) to shorten or become more compact as the concavity of the skull increases. Finally, those linear distances from nape through vertical pectoral [characters (24)–(25)] decrease or shorten as distances from vertical pectoral through mid-point between vertical pectoral and dorsal origin [characters (26)–(27)] increase. These transformations essentially describe the vertical development of the nuchal hump; they indicate that distances immediately preceding the hump

decrease or shorten to form a ramp, whereas distances involved directly with the hump itself lengthen or increase.

Size-related shape changes in the head and nuchal region of *G. cypha* are complex. Comparisons of the results from PCA and sheared PCA (PC2 and H2, Table 1) suggested that the anterodorsal hump and its associated structures vary complexly as growth proceeds, with some characters increasing in magnitude while others decrease. How ontogeny influences the onset and development of this hump is still to be elucidated. However, sexual differences in the degree of nuchal development can clearly be ruled out as one hypothesis pertaining to the origin and/or maintenance of this unusual structure.

ACKNOWLEDGMENTS

Special thanks to D. A. Hendrickson (University of Texas, Austin–UT) and C. O. Minckley (Northern Arizona University–NAU, Flagstaff) who organized and executed the Arizona Game and Fish Monitoring Program for humpback chub (1987–90) and who invited my participation in the project. During this period, many individuals provided me with assistance in the canyon: B. Bagley, C. Lutz, D. Papoulias, D. Valenciano, R. Van Haverbeke (all of AZGF), and P. Ryan and J. Nystadt (Navajo Natural Heritage Program). Grand Canyon National Park kindly allowed research within the park's boundaries. GCNP and AZGF provided permits to collect fish at the confluence, and Navajo Game and Fish supplied a collecting permit for the upstream Little Colorado River. The United States Fish and Wildlife Service allowed me to engage in endangered fish research as a sub-permitee under Federal Permit 676811. The cooperation and assistance of all of these organizations and agencies is appreciated. The biology of *Gila* in general, and humpback chub in particular, was discussed earnestly and often with W. L. Minckley, P. C. Marsh, T. E. Dowling, and B. D. DeMarais (Arizona State University), C. O. Minckley (NAU), D. A. Hendrickson (UT), and D. Kubly (AZGF). Manuscript reviews were graciously provided by W. L. Minckley, P. C. Marsh, C. O. Minckley, T. E. Dowling, K. M. Somers, W. J. Rainboth, and three reviewers. This research was supported by NSF-BSR-87-14552. Manuscript preparation was subsidized by BOR-1-FC-90-10490 and BOR-1-SP-40-0970A. Donations by Intel Corporation (Hillsboro, Oregon) to the endangered species program of MED are also acknowledged.

LITERATURE CITED

- AIROLDI, J.-P., AND B. K. FLURY. 1988. An application of common principal component analysis to cranial morphometry of *Microtus californicus* and *M. ochrogaster* (Mammalia, Rodentia). *J. Zool. (London)* 216:21-36.
- BOOKSTEIN, F., B. CHERNOFF, R. ELDER, J. HUMPHRIES, G. SMITH, AND R. STRAUSS. 1985. Morphometrics in evolutionary biology. *Acad. Nat. Sci. Philadelphia, Spec. Publ.* 15.
- CARLSON, C. A., AND R. T. MUTH. 1989. The Colorado River: lifeline of the American southwest. *Can. Fish. Aquat. Sci., Spec. Publ.* 106:220-239.
- COLLETTE, B. B. 1990. Problems with gray literature in fishery science, p. 27-31. *In: Writing for fishery journals.* J. R. Hunter (ed.). American Fisheries Society, Bethesda, Maryland.
- DIXON, W. J. (ED.). 1990. BMDP statistical software manual, Vol. 1. Univ. of California Press, Los Angeles.
- 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.
- , G. D. SCHNELL, AND D. J. HOUGH. 1986. Variation in spinner dolphin (*Stenella longirostris*) from the eastern tropical Pacific Ocean: sexual dimorphism in cranial morphology. *J. Mammal.* 67:537-544.
- HARRIS, R. J. 1975. A primer of multivariate statistics. Academic Press, New York, New York.
- HOLDEN, P. B. 1991. Ghosts of the Green River: impacts of Green River poisoning on management of native fishes, p. 43-54. *In: Battle against extinction: native fish management in the American west.* W. L. Minckley and J. E. Deacon (eds.). Univ. of Arizona Press, Tucson.
- , AND W. L. MINCKLEY. 1980. *Gila cypha* Miller, Humpback chub, p. 163. *In: Atlas of North American freshwater fishes.* D. S. Lee, C. R. Gilbert, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. R. Stauffer, Jr. (eds.). North Carolina State Museum of Natural History, Raleigh.
- , AND C. B. STALNAKER. 1970. Systematic studies of the cyprinid genus *Gila* in the upper Colorado River Basin. *Copeia* 1970:409-420.
- HOTELLING, H. 1933. Analysis of a complex of statistical variables into principal components. *J. Educ. Psychol.* 24:417-441.
- JOLICOEUR, R., AND J. E. MOSIMANN. 1960. Size and shape variation in the painted turtle: a principal component analysis. *Growth* 24:299-354.
- KAEDING, L. R., AND M. A. ZIMMERMAN. 1983. Life history and ecology of the humpback chub in the Little Colorado and Colorado Rivers of the Grand Canyon. *Trans. Am. Fish. Soc.* 112:577-594.
- , 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. *Ibid.* 119:135-144.
- MCCARTHY, M. S., AND W. L. MINCKLEY. 1987. Age-estimation for razorback suckers (Pisces: Catostomidae) from Lake Mojave Arizona-Nevada. *J. AZ-NV Acad. Sci.* 21:87-97.
- MILLER, R. R. 1946. *Gila cypha*, a remarkable new species of cyprinid fish from the Colorado River in Grand Canyon, Arizona. *J. Washington Acad. Sci.* 36:409-415.
- . 1955. Fish remains from archaeological sites in the lower Colorado River Basin, Arizona. *Pap. Michigan Acad. Sci., Arts and Letters* 40:125-136.
- . 1959. Origin and affinities of the freshwater fish fauna of western North America, p. 187-222. *In: Zoogeography.* C. L. Hubbs (ed.). American Assoc. for the Advancement of Sci., Publ. 51. Washington, D.C.
- . 1961. Man and the changing fish fauna of the American southwest. *Pap. Michigan Acad. Sci., Arts and Letters* 46:365-404.
- , AND G. R. SMITH. 1984. Fish remains from Stanton's Cave, Grand Canyon of the Colorado, Arizona, with notes on the taxonomy of *Gila cypha*, p. 61-65. *In: The archaeology, geology, and paleobiology of Stanton's Cave, Grand Canyon National Park, AZ.* R. C. Euler (ed.). Grand Canyon Natural History Assoc., Monogr. 6.
- MINCKLEY, W. L. 1973. Fishes of Arizona. Arizona Game and Fish Department, Phoenix, Arizona.
- , AND M. E. DOUGLAS. 1991. Discovery and extinction of western fishes: a blink of the eye in geologic time, p. 7-17. *In: Battle against extinction: native fish management in the American west.* W. L. Minckley and J. E. Deacon (eds.). Univ. of Arizona Press, Tucson.
- , D. A. HENDRICKSON, AND C. A. BOND. 1986. Geography of western North American freshwater fishes: description and relationships to intracontinental tectonism, p. 519-613. *In: Zoogeography of North American freshwater fishes.* C. H. Hocutt and E. O. Wiley (eds.). John Wiley and Sons, New York, New York.
- RINNE, J. N., J. E. JOHNSON, B. L. JENSEN, A. W. RUGER, AND R. SORENSEN. 1986. The role of hatcheries in the management and recovery of threatened and endangered fishes, p. 271-285. *In: Fish culture in fisheries management.* R. H. Stroud (ed.). American Fisheries Society, Bethesda, Maryland.
- RISING, J. D., AND K. M. SOMERS. 1989. The measurement of overall size in birds. *The Auk* 106:666-674.
- ROHLF, F. J., AND F. L. BOOKSTEIN. 1987. A comment on shearing as a method of "size correction." *Syst. Zool.* 36:356-367.
- ROHWER, S. A., AND D. L. KILGORE, JR. 1973. Interbreeding in the arid land foxes, *Vulpes velox* and *V. macrotis*. *Ibid.* 22:157-165.
- ROLSTON, H., III. 1991. Fishes of the desert: paradox and responsibility, p. 93-108. *In: Battle against extinction: native fish management in the American west.* W. L. Minckley and J. E. Deacon (eds.). Univ. of Arizona Press, Tucson.
- SAS INSTITUTE INC. 1985. SAS user's guide: statistics, Ver. 5 Edition. Statistical Analysis Systems Institute, Inc., Cary, North Carolina.
- SCHNELL, G. D., G. L. WORTHEN, AND M. E. DOUGLAS. 1985. Morphometric assessment of sexual dimor-

- phism in skeletal elements of California gulls. *The Condor* 87:484–493.
- 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. Nat. Park Serv. Trans. Proc., Ser. 5:613–623.
- STRAUSS, R. M., AND F. L. BOOKSTEIN. 1982. The truss: body form reconstruction in morphometrics. *Syst. Zool.* 31:113–135.
- SUTTKUS, R. D., AND G. H. CLEMMER. 1977. The humpback chub, *Gila cypha*, in the Grand Canyon area of the Colorado River. Occ. Pap. Tulane Univ. Mus. Nat. Hist. 1:1–30.
- THORPE, R. S. 1983. A review of the numerical methods for recognizing and analyzing racial differentiation, p. 404–423. *In*: Numerical taxonomy. J. Felsenstein (ed.). Springer-Verlag, New York, New York.
- . 1988. Multiple group principal component analysis and population differentiation. *J. Zool. (London)* 216:37–40.
- TITUS, K., J. A. MOSHER, AND B. K. WILLIAMS. 1984. Chance-corrected classification for use in discriminant analysis: ecological applications. *Amer. Midl. Natur.* 111:1–7.
- TYUS, H. M., B. D. BURDICK, R. A. VALDEZ, C. M. HAYNES, T. A. LYTLE, AND C. R. BERRY. 1982. Fishes of the upper Colorado River Basin: distribution, abundance and status, p. 12–70. *In*: Fishes of the upper Colorado River system: present and future. W. H. Miller, H. M. Tyus, and C. A. Carlson (eds.). West. Div., Am. Fish. Soc., Albuquerque, New Mexico.
- WILBUR, R. L. 1990. Gray literature: a professional dilemma. *Fisheries* 15:2–6.
- WYLBUR, H. M., D. W. TINKLE, AND J. P. COLLINS. 1974. Environmental certainty, trophic level, and resource availability in life history evolution. *Am. Nat.* 108:805–817.
- DEPARTMENT OF ZOOLOGY AND MUSEUM, ARIZONA STATE UNIVERSITY, TEMPE, ARIZONA 85287-1501. Submitted 18 Feb. 1992. Accepted 2 Aug. 1992. Section editor: D. G. Buth.

Copeia, 1993(2), pp. 343–351

Effects of Vitamin C Deficiency on Body Shape and Skull Osteology in *Geophagus brasiliensis*: Implications for Interpretations of Morphological Plasticity

PETER H. WIMBERGER

Morphological plasticity in fish and other vertebrates is usually attributed to the bone and muscle remodelling that results from exposure to different environmental regimes requiring different kinematic responses, such as feeding on different diets. I examined the extent of morphological plasticity that could be induced by feeding *Geophagus brasiliensis*, fish known to respond via plasticity to different foods, diets identical in consistency, but which differed in the amount of vitamin C they contained. Vitamin C is crucial to proper bone and connective tissue development, and there are numerous qualitative reports of deformation related to vitamin C deficiency. In this experiment, fish fed the vitamin C deficient diet differed in caudal peduncle measurements and had shorter snout and oral jaw measurements than fish fed the vitamin C sufficient diet. These results suggest that both nutritional differences between diets and mechanics of feeding on different diets in different ways can induce morphological plasticity.

THERE are a growing number of reports of phenotypic plasticity of jaw, skull, and body shape in vertebrates (Collins and Cheek, 1983; Witte, 1984; Wimberger, 1991). Those that involve trophic morphology usually infer that differences in the way that an organism captures or processes prey lead to the induced morphological differences. A heretofore unexplored alternative explanation for the morphological differences is that differences in nutrition between diets lead to morphological

differences. In this paper, I examine the effect of vitamin C deficiency on jaw, skull, and body shape in *Geophagus brasiliensis*, a species known to be morphologically plastic when fed different diets (Wimberger, 1991, 1992), to determine whether the morphological differences were due to feeding behavior or nutritional effects.

Differences in both body shape and jaw and skull morphology were previously induced in two species of the cichlid genus *Geophagus* by feeding juveniles either chironomid larvae or