

Morphometrics for the Determination of Sex and Hybridization in *Hypophthalmichthys* Species

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Introduction

Bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*) apparently hybridize readily in the wild in the United States. We first noticed the presence of *Hypophthalmichthys* hybrids in 2003. In the summer of 2005, five percent of *Hypophthalmichthys* we captured in the Missouri River were nominal hybrids. Hybrids can be difficult to identify because they can closely resemble either of the parent species (Fig. 1), and because the hybrids are fertile and backcrosses may exist. In 2003, in a preliminary study of 13 fish (2 each silver and bighead carp and 9 hybrids), gill raker morphology (Fig. 2) was 100% accurate in determining hybridization status, as verified by genetic analysis, but the small sample size of that study leaves need for further determination of the effectiveness of that technique for determination of hybridization. Incorrect identification of hybrids can compromise research findings. Tracking the rate of occurrence of hybrids is also important to understand population trends and the effect of the presence of *Hypophthalmichthys* hybrids on North American ecosystems.

Some authors have had success in identification of the sex of *Hypophthalmichthys* using ridges or tubercles on the pectoral fins (Freeze and Henderson 1982) but we have found that those characteristics are not reliable indicators during periods when the fish are not sexually active. For telemetry studies in which tags are implanted outside the spawning season, it is desirable to know the sex of the tagged fish.

Methods

Fish were captured by trammel nets from the Missouri River in central Missouri. All nominal bighead and silver carp were captured in December 2004 or January 2005. Some nominal hybrids were captured in that period and some additional hybrids were captured in summer 2006. All fish were of adult size. Total length (TL) of silver carp included in the study was 647 to 895 mm ($\sigma = 40$). Bighead carp measured 623 to 835 mm ($\sigma = 44$). Species and hybridization status were nominally determined by gill raker morphology (Fig. 2). Fish with fused, spongelike gill rakers with a smooth, unbroken anterior margin were considered to be silver carp, and fish with straight, comblike gill rakers were considered bighead carp. Any other morphology was considered indicative of a hybrid. Sex was determined by incision and examination of gonads. We measured eight external morphometric parameters (Table 1) on 76 female and 55 male bighead carp, 64 female and 40 male silver carp, and 11 male and 3 female hybrids. We also measured gut length on 13 bighead carp, 12 silver carp, and 14 hybrids. Parameters used for statistical analysis were ratios of two measurements. A total of 21 different ratios of external features, plus gut length:TL were examined for potential use (Table 1). We compared the ranges of the different ratios between different species/hybrids and sexes of both species looking for amount of overlap between different groups, and used ANOVA to analyze for statistical differences between the different groups. Discriminate analysis was used to determine which ratio or combination of ratios was most effective in discriminating the different groups. The gut length:TL ratio was not included in the discriminate analysis. A student's T-test ($\alpha = 0.05$) was used to test for differences in the different ratios between species or sexes.

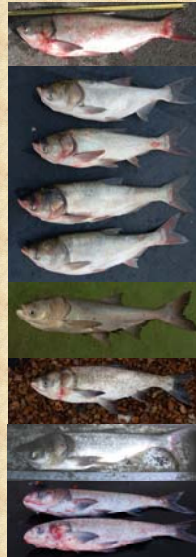


Figure 1. Can you identify the hybrids?



Figure 2. A & B: Hybrid gill rakers. C: Comparison of *Hypophthalmichthys* gill rakers 1: Bighead carp, 2: Hybrid, 3: Silver carp.

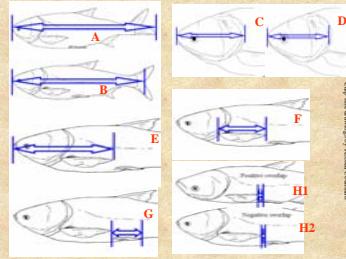


Figure 3. External parameters measured. A: total length, B: fork length, C: snout to soft opercle, D: snout to hard opercle, E: snout to pectoral fin tip, F: length of pectoral fin, G: length of pelvic fin, and H: overlap of pectoral and pelvic fin.

Results

Determination of sex

Most external morphometric ratios tested were significantly different between males and females of bighead and silver carps (Table 1). Discriminate analysis found two useful morphometric ratios for determination of sex in bighead carp. These were pectoral fin length:TL ($F = 52.5$) and snout to hard opercle distance: fork length:TL ($F = 21.2$). There was substantial overlap between males and females for both of these parameters (Fig. 4a), but by using both of these parameters, the analysis was able to correctly assess sex in 81% of females and 76% of males (Table 2). Discriminate analysis found three useful ratios for determination of sex in silver carp (pectoral fin length:TL, $F = 13.9$, overlap:FL, $F = 11.6$, and overlap:TL, 11.3). There was substantial overlap between males and females for all of these parameters, (Fig. 4b), but by using all three of these parameters, the analysis was able to correctly assess sex in 86% of females and 77% of males (Table 2).

Determination of Hybridization

There was a large difference in gut length:TL ratios between nominal bighead and silver carp, and gut length:TL ratios of all but two nominal hybrids were lower than the lowest value for silver carp and higher than the highest value for bighead carp (Fig. 5). However, gut length:TL of two nominal hybrids fell well within the range for silver carp.

All examined external morphometric ratios were significantly different between nominal bighead and silver carp (Table 1), but the ranges of values overlapped between the species for most ratios. The ranges of the ratios of pectoral-pelvic fin overlap and total length (TL), fork length (FL), pelvic fin length and pectoral fin length did not overlap, but even in these cases, the differences between the minimum ratio for bighead carp and the maximum for silver carp was not large (Table 1). Mean values for nominal hybrids were always intermediate to those of silver and bighead carps, but usually fell within the range of values seen for bighead carp.

Three external morphometric ratios were selected by the discriminate analysis for determination of hybridization. These were overlap:pectoral fin length ($F = 682$), snout to soft opercle: TL ($F = 89.1$), and pectoral fin length:FL ($F = 5.29$). Using all three ratios selected by discriminate analysis for identification of hybrids, the analysis agreed with the nominal determination from gill raker morphology in all cases for silver carp, but sometimes disagreed in discrimination of hybrids and bighead carp (11% of cases, for bighead and hybrids combined, (Table 3). Three nominal bighead carp were strongly identified as hybrids by the discriminate analysis of morphometric parameters ($p = 0.842$, 0.852, and 0.987) and two nominal hybrids were strongly identified as a bighead carp ($p = 0.999$). Notably, both of these fish had gut length:TL ratios higher than any nominal bighead carp (6.61 and 6.96), falling well into the hybrid range.

Table 1. Means of morphometric ratios used in discriminate analysis, and results of Student's T-tests comparing sexes and bighead versus silver carps

	Fork : Total	SHO ¹ : Fork	SBO ² : Total	SSO ³ : Fork	SSO ³ : Total	Pectoral fin : Fork	Pectoral fin : Total	Snout to pec. fin : Fork	Snout to pec. fin : Total	Pectoral fin : SHO ¹	Pectoral fin : SSO ³	Pec/Pec overlap ⁴ : Fork	Pec/Pec overlap ⁴ : Total	Pec/Pec overlap ⁴ : SHO ¹	Pec/Pec overlap ⁴ : SSO ³	Pelv. fin : Total	Pelv. fin : SHO ¹	Pelv. fin : SSO ³	Pelv. fin : SHO ¹ : SSO ³	Gut : Total	
Bighead carp	0.896 (0.001)	0.289 (0.002)	0.258 (0.001)	0.319 (0.002)	0.285 (0.002)	0.215 (0.002)	0.192 (0.001)	0.489 (0.002)	0.428 (0.007)	0.745 (0.006)	0.675 (0.006)	0.060 (0.002)	0.054 (0.008)	0.231 (0.011)	0.372 (0.011)	0.159 (0.001)	0.142 (0.005)	0.714 (0.004)	0.551 (0.004)	0.500 (0.004)	4.400 (0.151)
Silver carp	0.889 (0.001)	0.236 (0.001)	0.210 (0.001)	0.256 (0.001)	0.227 (0.001)	0.189 (0.001)	0.168 (0.001)	0.416 (0.002)	0.370 (0.006)	0.803 (0.006)	0.742 (0.004)	-0.004 (0.002)	-0.004 (0.003)	-0.024 (0.011)	-0.031 (0.009)	0.145 (0.001)	0.129 (0.007)	0.763 (0.004)	0.613 (0.008)	0.567 (0.007)	8.30 (0.218)
Bighead vs Silver	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001
Bighead vs Silver T-test p value	0.538	0.002	0.001	0.083	0.027	-0.001	-0.001	0.104	0.103	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	0.146	0.108	-0.001	0.001	0.004	0.415
Silver vs Silver	0.714	0.054	0.124	0.009	0.005	-0.001	-0.001	0.002	0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	0.010	0.009	0.530	0.006	0.001	0.709

¹snout to hard opercle distance; ² snout to soft opercle distance; ³ overlap of pectoral and pelvic fins

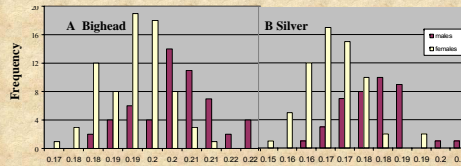


Figure 4. Pectoral fin-pelvic fin overlap : TL ratios for bighead (A) and silver (B) carps, comparing males to females

Table 2. Discriminate analysis prediction of sex from morphometrics versus actual sex. Correct prediction in red.

	Classified into:	
	Female	Male
Bighead Carp Female	59 (80.8%)	14 (19.8%)
Bighead Carp Male	13 (23.6%)	42 (76.4%)
Silver Carp Female	54 (85.7%)	9 (14.3%)
Silver Carp Male	9 (23.1%)	30 (76.9%)

Table 3. Discriminate analysis prediction of species from morphometric data versus nominal determination from gill raker morphology. Agreement in red.

	Classified into:		
	Bighead	Hybrid	Silver
Nominal Bighead	115 (89.84%)	13 (10.16%)	0
Nominal Hybrid	2 (15.38)	11 (84.62%)	0
Nominal Silver	0	0	102 (100%)

Discussion

Determination of sex

External morphometrics generally varied with the sex of *Hypophthalmichthys* spp. collected outside the breeding season. However, there was no single parameter that could be conclusively used to determine sex in the field. When two (for bighead carp) or three (for silver carp) parameters were combined, discriminate analysis was successful in determining sex in 76 – 86% of individuals.

Hybridization

Hypophthalmichthys hybrids are often used in aquaculture (Kolar and others, in press), and F1 hybrids have been shown to have a substantially higher growth rate than either parent species. These hybrids are reproductively fertile and introgression is considered a problem in aquaculture (Mia and others 2005). Hybridization in the wild is thought to be rare within the native range (Yi and others 1988; Mat 2003), but Verigin and others (1979) noted a high degree of hybridization between Bighead and Silver carps in the Syr'Darya River (Kazakhstan, Tajikistan, and Uzbekistan) where the fish are introduced. Those authors speculated that eventually pure strains of both species might become rare in that system, but no recent information is available. If hybridization and introgression become common in the United States, these factors will influence models generated for the control of invasive *Hypophthalmichthys* spp. Also, as researchers study the ecology of these fishes in the United States, the failure to recognize these hybrids may compromise research data.

In this study, all nominal hybrids (as determined by gill raker morphology) either had gut lengths longer than any bighead carp or shorter than any silver carp, or were classified as hybrids by the discriminate analysis of external morphometrics. Ten of fourteen nominal hybrids were classified as hybrids by both of these alternate criteria. Thus, these data strongly support the hypothesis that intermediate or abnormal gill raker morphology is a good indicator of hybridization. However, it is unknown if all hybrids and backcrosses will present abnormal gill raker morphology. The discriminate analysis assigned three nominal bighead carp a probability of greater than 80% of being hybrids, and one of these a probability of 99%. There is a reasonable chance that these fish were hybrids or backcrosses that were not identified by their gill raker morphology. If *Hypophthalmichthys* populations in the United States are or become substantially introgressed, identification of hybrids will become more difficult.

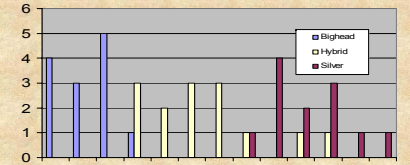


Figure 5. Gut length : Total Length ratios for bighead, hybrid, and silver carps

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