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Morphological changes of silver and bighead carp in the Yangtze River over the past 50 years

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Abstract: Multivariate analysis was adopted to analyze 30 morphometrical characteristics of 121 one-year-old juvenile silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) bred during the 1950s ("the former population") and 2008 ("the current population") and collected from the middle reach of the Yangtze River. The average discriminant accuracies of the former and current silver and bighead carp population were 94.2% and 98.0%, respectively. Discriminant analysis also revealed that significant differences in morphology occurred between the former and current populations of both carp in overall characteristics. One-way analysis of variance indicated that between former and current populations, silver carp showed highly significant differences (P<0.01) in twelve of their characteristics and significant differences (P<0.05) in eight of their characteristics, while bighead carp showed highly significant differences (P<0.01) in eight of their characteristics and significant differences (P<0.05) in eight of their characteristics. Six head morphology variables of the current silver and bighead carp were significantly or highly significantly larger than the former populations; fourteen characteristics of silver carp and ten characteristics of bighead carp of the current populations, mainly reflecting truck and tail morphology, were significantly or very significantly smaller than the former populations. Our results indicate that silver and bighead carp have developed a larger head and smaller truck and tail during the last 50 years. Due to such morphological changes, it seems apparent that the heads of these fish species need to be considered in regards to human diets, particularly in relation to economic and nutritious value.

Key words: Silver carp; Bighead carp; 50 years breeding; Morphological change; Yangtze River

50 年来长江鲢、鳙形态特征的变迁

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摘要：采用多元分析方法, 对采自长江中游的上世纪中期(下称早期)和本世纪初(下称当前)的 121 尾 1 龄鲢、鳙的 30 项形态度量性状进行了分析。依据对判别贡献最大的 5 个参数所建立的判别公式, 对鲢、鳙早期和当前群体的平均判别准确率分别达 94.2%和 98.%, 显示鲢或鳙的当前与早期群体间在总体上存在着显著差异。单因素方差分析显示, 在早期和当前群体间, 鲢有 12 个特征差异极显著、8 个差异显著, 鳙分别有 8 个特征差异极显著或显著。其中, 鲢和鳙的 6 个显著或极显著变大的特征参数集中在头部, 而 14 个鲢和 10 个鳙的显著或极显著变小的特征参数则主要集中在躯干部与尾部。研究表明, 最近 50 年来长江鲢、鳙的头及头部特征有相对变大的趋势, 而躯干部与尾部的许多特征参数则有相对变小的趋势, 这些形态演变趋势符合人们喜食鱼头、追求高经济效益的期望。

关键词：鲢; 鳙; 人工繁殖 50 年前后; 形态变迁趋势; 中国长江
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As early as the Tang Dynasty, silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) have been the main type of fresh water fish cultivated in China. Together with grass carp (*Ctenopharyngodon idellus*) and black carp (*Mylopharyngodon piceus*), they are considered China's major carp species. Following the successful breeding of these species during the late 1950s, cultivation of silver and bighead carp has been introduced to 80 countries and 68 regions around the world and they now have the highest aquaculture output in China and globally (Schofield et al, 2005). The Yangtze River, a high quality gene pool of the four major Chinese carp, has attracted significant scientific attention in regards to the biological characteristics of silver and bighead carp and its resource status (Survey Team of Spawning Grounds of Domestic Fishes in Yangtze River, 1982; Li et al, 1995; Liu et al, 1992; Chen et al, 1995; Liu et al, 1997, 2004; Qiu et al, 2002). In the last three decades, however, natural fishery resources have been seriously damaged as a result of excessive fishing, water pollution, and hydro project construction. Additionally, large groups of farmed fish have been released into the Yangtze River, which has disrupted this natural gene bank. To protect such fishery resources, it is important to track the genetic conditions of silver and bighead carp in the Yangtze River, as well as changes in their biological characteristics (Cao, 2008).

A number of intensive studies on the morphological and growth characteristics of silver and bighead carp have been conducted in China, which have identified the morphological standards for their original populations (Li et al, 1989, 1990, 1995; Liu et al, 1992; Sun et al, 1992; Ding et al, 2003; Yu et al, 2009). As the oldest and simplest way to detect quantitative characteristics controlled by polygenes, morphometry not only reflects genetic conditions but also cultured capability. Based on a good understanding of the phenotypic changes of silver and bighead carp during their ontogenetic process (Yu et al, 2010), we measured the external morphological characteristics of one-year-old silver and bighead carp collected during the mid 20th century and early 21st century to reveal the morphological variation trends of these Yangtze River carp after 50 years of successful breeding. This was achieved through multivariate morphometrics to provide a basis to evaluate their genetic changes.

1 Materials and Methods

1.1 Materials

The former population was comprised of specimens collected during the 1950s by the Institute of Hydrobiology, Chinese Academy of Sciences and deposited in the Freshwater Fish Museum, which included 20 silver carp and 17 bighead carp. The current population was collected in 2008 from the National Laojianghe Primary Breeding Farm for the Four Domesticated Fishes (29°35′N, 113°00′E) in Jianli County, Hubei Province, which included 32 silver carp and 42 bighead carp. All specimens were undamaged and possessed a normal body shape. The specimens were preserved in 10% formalin solution. All fish for the two species were one year old, with age determined for the current specimens from scales below of dorsal fin and above the lateral line and for historical specimens based on similar size ranges to the current specimens (Tab. 1).

Tab. 1 Number and size of *Hypophthalmichthys molitrix* and *Aristichthys nobilis*

	Population	Sampling locality	Sampling time	Number	Body length(cm)		Body weight(g)	
					Range	Mean	Range	Mean
Hypophthalmichthys molitrix	Former population	Danjiang River	1957	1	20	20	177.9	177.9
		Hong Lake	1959	1	17	17	162.7	162.7
		Laohekou inYunxian	1957	1	26	26	333.1	333.1
		Poyang Lake	1959	5	17.6−25.8	21.16	98.6−430.5	200.54
		Liangzi Lake	1955	12	16−29	21.75	74.4−595.1	233.04
	Current population	Laojianghe Lake	2008	32	15−26	20.73	66−512	182.36
Aristichthys nobilis	Former population	Danjiang River	1973	4	10.2−15.7	12.9	187−679	42.025
		Five Lake in Hanyang	1975	1	21	21	172.1	172.1
		Dong Lake	1956	8	13.2−39.3	27.6	353−12237	569.325
		Laohekou inYunxian	1973	1	35	35	874.7	874.7
		Chongming Island	1957	1	26.4	26.4	366.4	366.4
		Poyang Lake	1959	2	15−27	21	68.7−415.7	242.2
	Current population	Laojianghe Lake	2008	42	18−39	25.10	158−1354	440.87

1.2 Morphometrical methods

Pictures were taken indoors. The lens was positioned perpendicular to the fish body at a distance four times as long as the length of fish body. The photos were then used as the measuring object. A total of 30 traditional characteristics and frame parameters were measured (Tab. 2). All data were obtained from the left side of the fish. Traditional characteristics measured included full body length, head length, proboscis length, eye orbit diameter, body depth, pectoral fin length, caudal peduncle length, caudal peduncle depth, and head depth. Ten anatomical coordinate points were selected as the measuring point for frame data (Fig. 1) (Bookstein et al, 1985; Li et al, 1990; Xie et al, 2003; Min et al, 2009) and a total of 21 frame data were built. All measurements were the distances between point to point, calculated from the pictures and measurement software and accurate to 0.01 mm.

Tab. 2 Morphometric characteristics taken on the specimen

Code	Measurement	Code	Measurement	Code	Measurement
1	Total length	2	Head length	3	Snout length
4	Eye diameter	5	Body depth	6	Pectoral fin length
7	Length of caudal peduncle	8	Depth of caudal peduncle	9	Depth of head
10	D_{1-2}	11	D_{2-3}	12	D_{3-4}
13	D_{4-5}	14	D_{5-6}	15	D_{6-7}
16	D_{7-8}	17	D_{8-9}	18	D_{9-1}
19	D_{1-10}	20	D_{2-10}	21	D_{9-10}
22	D_{2-9}	23	D_{3-8}	24	D_{4-7}
25	D_{2-8}	26	D_{3-9}	27	D_{3-7}
28	D_{4-8}	29	D_{6-7}	30	D_{4-5}

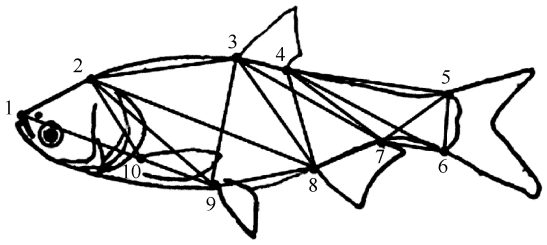

Fig. 1 Schematic measurement for truss network

1, Snout tip; 2, Most anterior scales on skull; 3, Origin of dorsal fin; 4, Back end of dorsal fin base; 5, Dorsal origin of caudal fin; 6, Ventral origin of caudal fin; 7, Back end of anal fin base; 8, Origin of anal fin; 9, Origin of pelvic fin; 10, Origin of pectoral fin. Truss parameter measurement is the distance between two of the ten landmark points. For example, D1-2 denotes the distance between landmark points 1 and 2.

1.3 Data analysis

Meristic measurements vary greatly in magnitude values. To weigh in measurements of smaller absolute values, snout length and eye diameter were standardized to head length and the remaining measurements were standardized to total body length. All data were first tabulated with Microsoft Excel, and then analyzed with SPSS 11.0 (Yu et al, 2003).

Discriminant functions were established using the stepwise method, and samples were categorized based on the discriminant functions. Function accuracy was then tested (Li et al, 1998).

One-way analysis of variance was used to test differences between groups.

2 Results

2.1 Discriminant analysis of overall differences in characteristics

Discriminant formulas for the former and current populations were established according to the nine measurable characteristics and twenty-one frame numbers after standardization, respectively. To facilitate discrimination, we selected five parameters from the thirty normalizing morphological parameters and established useful discriminant formulas. The five parameters that best discriminate silver carp are X_7, X_9, X_{10}, X_{12}, and X_{13}. The corresponding determinant formula is as follows:

For the former population:

$$Y=1186.552X_7+2753.702X_9+1763.454X_{10}+973.313X_{12}+1698.968X_{13}-987.140$$

For the current population:

$$Y=1259.417X_7+2577.935X_9+2047.336X_{10}+836.615X_{12}+1588.906X_{13}-967.251$$

The five parameters that best discriminate bighead carp are X_2, X_3, X_8, X_{25}, and X_{30}. The corresponding discriminant formula is as follows:

For the former population:

$$Y=-256.895X_2+5.099X_3+583.569X_8+2457.615X_{25}+573.452X_{30}-738.916$$

For the current population:

$$Y=-108.136X_2+87.138X_3+497.113X_8+2301.423X_{25}+473.913X_{30}-680.064$$

The subscripts of the variables are the codes of characters.

The discriminant results are found in Tab. 3 and Tab. 4, which show that discriminant accuracy of the former silver carp population was 95.0% and the discriminant accuracy of the current silver carp population was 93.8%; and the discriminant accuracy of the former bighead carp population was 95.0% and the discriminant accuracy of

Tab. 3 Discriminant analysis of the two *Hypophthalmichthys molitrix* populations (based on five selected parameters)

Population	Number	Accuracy (%)	Predicted classification	
			Former population	Current population
Population last century	20	95.0	19	1
Population this century	32	93.8	2	30
Total	52	94.2	21	31

Tab. 4 Discriminant analysis of the two *Aristichthys nobilis* populations (based on five selected parameters)

Population	Number	Accuracy (%)	Predicted classification	
			Former population	Current population
Population last century	19	94.7	18	1
Population this century	31	100.0	0	31
Total	50	98.0	21	31

the current bighead carp population was 93.8%. The discriminant effects of both groups were very significant (P<0.01). Such results indicate significant differences between the former and current populations of silver and bighead carp in their overall characteristics.

2.2 One-way analysis of variance of Specific Character Changes

To determine which characteristics experienced specific changes between the current and former populations, we conducted single-factor variance analysis of the 30 parameters used to discriminate the two population groups (Tab. 5 and Tab. 6). Results demonstrated that between former and current populations, silver carp showed highly significant differences (P<0.01) in twelve characteristics and significant differences (P<0.05) in eight characteristics. Among all characteristic parameters, six representing head morphology (head length, head back length, snout length, eye orbit diameter, tip of snout, and starting point of pectoral fins) became larger, while fourteen representing length and depth of trunks and tails become smaller.

Between former and current populations, bighead carp showed highly significant differences (P<0.01) in eight and significant differences (P<0.05) in eight of their characteristic parameters. Among all characteristic

Tab. 5 Characteristics of high variance between the two *Hypophthalmichthys molitrix* populations

Characteristic	Mean values(cm)		Significance level	Trend of change
	Former population	Current population		
Head length/Body length	0.264±0.016	0.281±0.087	**	Bigger
Eye diameter/Head length	0.166±0.019	0.182±0.020	**	Bigger
$D_{1\text{-}2}$/Body length	0.216±0.011	0.233±0.009	**	Bigger
$D_{10\text{-}1}$/Body length	0.266±0.021	0.287±0.010	**	Bigger
$D_{1\text{-}9}$/Body length	0.468±0.018	0.486±0.013	**	Bigger
Snout length/ Head length	0.166±0.035	0.183±0.019	*	Bigger
$D_{2\text{-}}$/Body length $_3$	0.317±0.016	0.305±0.010	**	Smaller
$D_{4\text{-}5}$/Body length	0.364±0.023	0.342±0.014	**	Smaller
$D_{5\text{-}6}$/Body length	0.130±0.010	0.123±0.006	**	Smaller
$D_{2\text{-}8}$/Body length	0.548±0.014	0.534±0.013	**	Smaller
$D_{3\text{-}7}$/Body length	0.380±0.022	0.366±0.013	**	Smaller
$D_{6\text{-}7}$/Body length	0.387±0.017	0.372±0.015	**	Smaller
$D_{3\text{-}8}$/Body length	0.332±0.028	0.317±0.009	**	Smaller
$D_{4\text{-}6}$/Body length	0.129±0.016	0.121±0.011	*	Smaller
$D_{7\text{-}8}$/Body length	0.164±0.014	0.157±0.009	*	Smaller
$D_{8\text{-}9}$/Body length	0.251±0.012	0.243±0.015	*	Smaller
$D_{4\text{-}7}$/Body length	0.284±0.018	0.274±0.014	*	Smaller
Depth of caudal peduncle/Body length	0.114±0.008	0.107±0.011	*	Smaller
$D_{3\text{-}9}$/Body length	0.300±0.02	0.289±0.008	*	Smaller
$D_{4\text{-}8}$/Body length	0.252±0.024	0.242±0.009	*	Smaller

**Difference was highly significant; *difference was significant.

Tab. 6 Characteristics of high variance between the two *Aristichthys nobilis* populations

Characteristic	Mean values(cm)		Significance level	Trend of change
	Former population	Current population		
Head length/Body length	0.318±0.029	0.351±0.017	**	Bigger
Snout length/ Head length	0.194±0.043	0.219±0.020	**	Bigger
Depth of head/Body length	0.253±0.016	0.264±0.014	**	Bigger
$D_{1\text{-}2}$/Body length	0.251±0.018	0.280±0.013	**	Bigger
$D_{9\text{-}1}$/Body length	0.467±0.017	0.468±0.012	**	Bigger
$D_{10\text{-}1}$/Body length	0.266±0.023	0.285±0.010	**	Bigger
$D_{4\text{-}5}$/Body length	0.337±0.024	0.312±0.022	**	Smaller
$D_{5\text{-}7}$/Body length	0.205±0.029	0.180±0.019	**	Smaller
Eye diameter/Head length	0.160±0.029	0.144±0.016	*	Smaller
Body depth/Body length	0.312±0.023	0.302±0.016	*	Smaller
$D_{6\text{-}7}$/Body length	0.387±0.016	0.371±0.016	*	Smaller
$D_{8\text{-}9}$/Body length	0.180±0.015	0.168±0.014	*	Smaller
$D_{2\text{-}10}$/Body length	0.112±0.102	0.103±0.012	*	Smaller
$D_{2\text{-}9}$/Body length	0.130±0.011	0.124±0.007	*	Smaller
Pectoral fin length/Body length/Body length	0.378±0.021	0.364±0.013	*	Smaller
$D_{2\text{-}3}$/Body length	0.251±0.023	0.238±0.013	*	Smaller

**Difference was highly significant；*difference was significant.

parameters, six representing head morphology (head length, head depth, head back length, snout length, tip of snout, and starting point of pectoral fins) and the starting point of pelvic fin became very significantly larger, while ten including eye orbit diameter, pectoral fin length, and length and depth of trunks and tails become smaller.

3 Discussion

The National Laojianghe Primary Breeding Farm is located in Jianli county of Hubei Province. The farm's water body was formed naturally in 1901 as a remnant lake on the north in Jianli Section, Middle Reaches of the Yangtze River. In 1958, dams were constructed at the entrance and exit of the water way, forming a narrow horseshoe lake 22.5 km long, 1.1 km wide, and encompassing a 49.4 km of shoreline. Under normal conditions, when the water level is 27.5 m, the water surface area is 1840 km^2, with the greatest water body depth of 19 m and average depth of 6 m. In 1991, the "Four Domesticated Fish Natural Germplam Resources Ecological Reserve of the Yangtze Water System" was established by the Ministry of Agriculture to protect these resources at the population level. Wild larva fish are collected from near the Yangtze River in May-June each year. The larvae are reared in the nursery pond until fish fry, when they were released into the lake without artificial feeding.

The largest silver or bighead carp living in the Yangtze River weigh up to 40 kg. The majority of silver carp become sexually mature at the age of three, while bighead carp become sexually mature at the age of four to five. Most parent fish breed in reaches possessing torrents and eddies, whilst young fish and already spawned parent fish tend to swim inhabit river bends and lakes for food and hibernation. During the process of growing from larva to adults, however, allometry occurs in some morphological characteristics (Osse et al, 1995) and overall features may change with individual ontogeny. According to our previous studies in the Yangtze River, significant allometry occurred in 73.3% of the morphological characteristics for silver carp between one and two years old and 56.7% for bighead carp between two and three years old. However, there was no significant allometry in the characteristic parameters of the same age group (Yu et al, 2010)

The specimens analyzed in this paper were all one-year old juveniles of similar body length, which allowed for natural differences arising from allometry to be eliminated. The average discriminant accuracies of former and current populations of silver and bighead carp were 94.2% and 98.0%, respectively, which indicates significant changes have taken place in the gross morphology of silver and bighead carp in the Yangtze River over the past 50 years. Single-factor analysis of variance indicated that between former and current populations, silver carp showed significant or very significant differences in 66.7% of their characteristics whereas bighead carp show significant or

very significant differences in 53.3% of their characteristics. Among all characteristics, six related to the head morphology of silver and bighead carp became significantly or very significantly larger, while fourteen related to the trunks and tails of silver carp and ten of bighead carp became significantly or very significantly smaller. These results show that during the past 50 years, the heads of silver and bighead carp in the Yangtze River have tended to increase in size, while their trunks and tails have tended to decrease in size.

These morphological changes reflect the possible differences of silver and bighead carp in genetic factors and living environment before and after breeding over the last 50 years. Though the biological significance of such changes in fish populations and their heredity mechanisms remain unknown, such changes can be considered desirable. Previous studies have found that silver and bighead carp heads are not only rich in EPA, DHA, and EAA but also contain higher contents of fat, calcium, and phosphor than their muscles (Li et al, 2008). The economic and nutritious value of silver and bighead carp heads is, therefore, much greater than of their trunks.

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