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Ontogenetic Changes in Meristic Measurements of Silver Carp and Bighead Carp

YU Hong-Xia, TANG Wen-Qiao*, LI Si-Fa

(*Laboratory of Ichthyology, Shanghai Ocean University, Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education*，*Shanghai* 200090, *China*)

Abstract: The study analyzed 30 exterior meristic characters for 255 silver and bighead carp samples of 1- to 5-year-old collected from the National Primary Breeding Farm at Laojianghe Lake at the Middle Reach of the Yangtze River. Multivariate analysis was performed. In silver carp, the Euclidean distance was the greatest between the 1-year-old group and other age groups. Silver carp individuals were correctly classified at 98.0% accuracy with a discriminant function established by discriminant analysis based on meristic measurements. Similarly, bighead carp had the greatest distance between 1- to 2-year-old group and other age groups. Individuals of bighead carp were correctly classified at 90.7% accuracy by the discrimination function. The data showed that morphological transformation occurred during the life history of silver and bighead carp development. Eighteen meristic measurements showed highly significant differences, while four showed a significant difference between the two silver carp groups. Ten parameters decreased, while twelve measurements increased during development. In bighead carp, fourteen parameters were significantly different, while three parameters were significantly different between the two groups. Twelve parameters were significantly decreased and another five were increased during development. The results suggest allometric growth should be taken into account when identifying species, analyzing population differences and establishing germplasm standards based on morphology.

Key words: *Hypophthalmichthys molitrix*; *Aristichthys nobilis*; Ontogeny; Morphology；Allometric growth；Yangtze River

长江鲢、鳙个体发育过程中的表型变化

于红霞，唐文乔*, 李思发

（上海海洋大学 鱼类研究室，水产种质资源发掘与利用教育部重点实验室 上海 201306）

摘要：采用多元分析方法，对采自长江老江河国家级四大家鱼原种场 255 尾 1—5 龄鲢、鳙的 30 项形态度量数据进行了分析。聚类分析显示，鲢可明显地聚成欧氏距离相对较远的 1 龄组和 2—5 龄组，鳙也可聚成欧氏距离较远的 1、2 龄组与 3—5 龄组。判别分析显示，144 尾鲢个体对所聚类的两个组别的判别准确率高达 98.0%，111 尾鳙个体对所聚类的两个组别的判别准确率也达 90.7%。这表明，在幼体向成体的发育过程中，鲢和鳙在形态上均存在着两个具有显著差异阶段。单因素方差分析显示，鲢两个组别之间有 18 个参数差异极显著，4 个差异显著，其中 10 个显著或极显著地变小，12 个显著或极显著地变大。鳙两个组别间有极显著和显著差异的参数分别有 14 个和 3 个，其中极显著或显著差异变小和变大的分别有 12 个和 5 个。这表明在鲢、鳙个体发育过程中，分别有 73.3%和 56.7%的特征参数具有生长异速现象。因此，在根据形态鉴别物种、分析种群差异、确立种质标准和作生长退算时，均应考虑所选用特征的生长异速现象。

关键词：鲢；鳙；个体发育；外部形态；生长异速；长江
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Silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) have been major fresh

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*Corresponding author (通讯作者), E-mail：wqtang@shou.edu.cn
First author: YU Hong-Xia（1982-），female，graduate student; E-mail:yuhongxia2002@163.com
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water aquaculture species since Tang Dynasty in China (Li et al, 1990). Since the success of artificial breeding of the four domesticated fishes during the 1950s, silver and bighead carp have been introduced to many countries world-wide (Schofield et al, 2005) and their farming yields were the highest in China and other countries (Li et al, 1990; Schofield et al, 2005). As the primary natural germplasm resources these two species are found along the Yangtze River and their biological characteristics and resource status in the Yangtze River have been further studied (Liu et al, 1992; Chen et al, 1995; Liu et al, 1997; 2004; Qiu et al, 2002). In recent 30 years, natural resources of silver and bighead carp have been severely damaged by water pollution, construction and over-fishing. The frequent escape and release of cultivated population to the wild have also disturbed the natural gene pool of these species. Monitoring silver and bighead carp germplasm resources in the Yangtze River and their biological variations has become an important task for resources protection (Liu et al, 1992, Cao et al, 2008).

Morphological characteristics and development of silver and bighead carp have been studied in great detail (Li et al, 1989, 1990, 1995; Liu et al, 1992; Sun et al, 1992, Ding et al, 2003; Yu et al, 2009). Morphological standards of primary breeding stock have been proposed (Li et al, 1997). As allometry exists in fish growth (Osse et al, 1995), it is necessary to study morphometrics of these species at various ages during their life cycle in order to evaluate germplasm quality and establish standards. However, no report has evaluated morphological changes from juveniles to adults of these two species.

In order to clarify the trend of morphological changes during juvenile to adult development, we have compared meristic characteristics of silver carp and big head of different ages from the Laojianghe Primary Breeding Farm. This information is necessary for the Yangtze River germplasm evaluation and the formulation of morphological standards for these primary resources.

1 Materials and Methods

1.1 Materials

The National Laojianghe Primary Breeding Farm for the Four Domesticated Fishes (29° 35′ N，113° 00′ E) is located in Jianli county, Hubei Province. The water body was formed naturally in 1901 as a remnant lake to the north bank of lower Jinjiang Section. In 1958, dams were constructed at the entrance and exit of the water way, forming a narrow horseshoe lake of 22.5 km long, 1.1 km wide with a 49.4 km shoreline. Normally, when water level is 27.5 m, the water surface area is 1840 km^2, with the greatest depth of 19 m and average depth of 6 m. The averaged annual rainfall of the lake is 1226 mm and 134 days, and the sunlight is 2004 h (Liu et al, 2002). 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 the fish population resource.

Samples were collected from the Primary Breeding Farm in January of 2008. One-year-old group were captured from the fry pond (6.7 hm^2) while other age groups were captured from the lake by net and picked at random with different sizes. Measurements of body length (accurate to 1 cm) and weight (accurate to 1 g) of each individual were taken on site. Photographs for each individual were taken from the left side by digital camera (Canon) at 1.2 m with visible scale. Intact scales (10-20 pieces) were taken from each individual and preserved in bags for age determination. Scales were submerged in water for 1-2 h, then cleaned and mounted between two slides. The slides were projected to screen for structure observation at 20 or 50× magnification. Age marks were determined as reported by Yu et al (2009). Ages, body length and weight of the fish were shown in Tab. 1 and Tab.2.

1.2 Body measurement

Digitized images of specimens were measured for 30 conventional and truss network measurements (Tab. 3). Conventional measurements include total length,

Tab. 1 Body length and weight of Silver Carp

Age group	No. of specimen	Body length (cm)		Body weight (g)	
		Range	Mean	Range	Mean
1	49	130-220	184.49	102-206	168.47
2	24	375-470	430.43	540-1750	1245.06
3	21	360-580	498.10	1240-3586	2470.65
4	34	460-600	526.47	1478-4300	3266.50
5	16	520-690	560.63	3012-4798	3976.24

Tab. 2 Body length and weight of Bighead Carp

Age group	No. of specimen	Body length (cm)		Body weight (g)	
		Range	Mean	Range	Mean
1	32	18-29	22.69	130-556	284.50
2	23	30-47	37.33	632-2534	1367.08
3	37	41-62	49.25	1240-5440	2811.18
4	15	44-70	57.40	1478-7000	4194.53
5	4	68-78	71.00	6000-10500	7800.00

head length, head depth, snout length, eye diameter, body depth, pectoral length, caudal peduncle length and depth. Ten morphological landmarks were used for truss network measurements, as shown in Fig. 1 (Li et al, 1990): tip snout, most anterior of scales on skull, origin of dorsal fin, back end of dorsal fin base, dorsal origin of caudal fin, ventral origin of caudal fin, back end of anal fin base, origin of anal fin, origin of pelvic fin, origin of pectoral fin. Twenty one measurements were constructed from these 10 landmark points. All truss measurements were directly obtained from the digital images using computer software (accurate to 0.01 mm).

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 rest of the 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).

Cluster analysis: Intergroup and mean intragroup Euclidean distances were first calculated, then

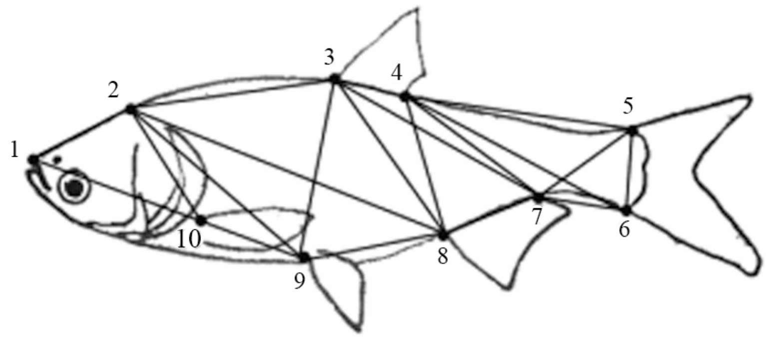

Fig. 1 Schematic measurement for truss network

1, Snout tip. 2, Most anterior of 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.

Note: Truss parameter measurement is the distance between two of the 10 landmark points. Forexample, D1-2 denotes the distance between landmark points 1 and 2.

Tab. 3 Morphometric characters

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}

hierarchical cluster analysis was performed using minimum distance method to create dendrograms (Zhang et al, 1982).

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

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

2 Results

2.1 Clustering analysis

Euclidean distances between groups were calculated from standardized conventional and truss measurements for silver and bighead carps (Tab. 4, 5). Hierarchical clustering dendrograms are shown in Fig. 2. Five age groups formed 3 clusters, in which cluster I contained only 1-year-old group, cluster II contained 2- and 3-year old-group, while cluster III contained 4- and 5-year-old group. However, distance between clusters II and III was relatively short. Bighead carp also formed 3 clusters, where cluster I contained 1- and 2-year-old-group, cluster II contained 3- and 4-year-old-group, while cluster III contained 5-year-old. Distance between clusters I and II was short too. These results suggest the development of these two species can be divided in two morphologically distinct stages.

2.2 Discriminant analysis

Discriminant functions were established stepwise for all clusters of silver carp and discriminant tests were performed. Results were shown in Tab. 6. The accuracy for cluster I discrimination was 98.0%. But the accuracy for cluster II and III was 64.4% and 78.0%. A high rate of misidentification occurred for individuals in clusters II and III. These results are consistent with the short distance between the two clusters. When clusters II and III are combined, where one-year-old group is still in cluster I, and the other age groups in cluster II, the discrimination accuracy is 98% (Tab. 7).

Similarly, discriminant analysis for bighead carp was highly accurate for cluster I, and less accurate for clusters II and III. After combining clusters II and III, discrimination accuracy was increased (Tab. 8). The

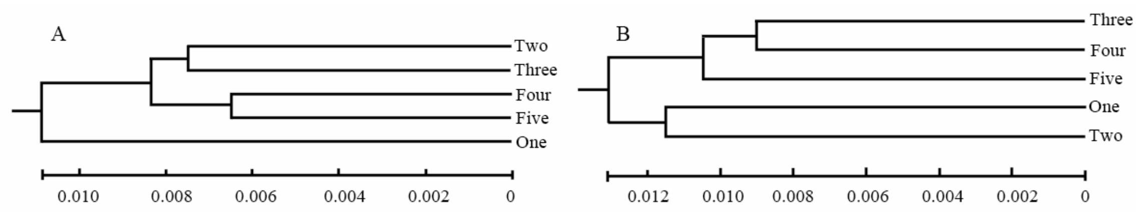

Fig. 2 Dendrogram of different age groups

A. Silver Carp; B. Bighead Carp.

Tab. 4 Euclidean distances among age groups of silver carp

Age group	No. of specimen	1	2	3	4	5
1	49	0.0000				
2	24	0.0168	0.0000			
3	21	0.0200	0.0146	0.0000		
4	34	0.0214	0.0159	0.0131	0.0000	
5	16	0.0293	0.0202	0.0179	0.0180	0.0000
Average distance within group		0.0087	0.0134	0.0127	0.0134	0.0185

Tab. 5 Euclidean distances among age groups of bighead carp

Age group	No. of specimen	1	2	3	4	5
1	32	0.0000				
2	23	0.0225	0.0000			
3	37	0.0266	0.0227	0.0000		
4	15	0.0282	0.0233	0.0182	0.0000	
5	4	0.0290	0.0268	0.0213	0.0211	0.0000
Average distance within group		0.0222	0.0214	0.0162	0.0201	0.0203

Tab. 6 Discrimination among three clusters of silver carp

Group	No. of specimen	Accuracy (%)	No. of specimen assigned by discriminant analysis		
			Group Ⅰ	Group Ⅱ	Group Ⅲ
Group Ⅰ	49	98.0	48	1	0
Group Ⅱ	45	64.4	2	29	14
Group Ⅲ	50	78.0	0	11	39
Total	144	80.56	50	41	53

Tab. 7 Discrimination between two combined clusters of silver carp

Group	No. of specimen	Accuracy (%)	No. of specimen assigned by discriminant analysis	
			Group Ⅰ	Group Ⅱ
Group Ⅰ	49	98.0	48	1
Group Ⅱ	95	98.0	2	93
Total	144	98.0	50	94

Tab. 8 Discrimination between two combined clusters of bighead carp

Group	No. of specimen	Accuracy (%)	No. of specimen assigned by discriminant analysis	
			Group Ⅰ	Group Ⅱ
Group Ⅰ	54	90.7	49	5
Group Ⅱ	64	90.6	6	58
Total	118	90.7	55	63

discrimination accuracy for the two clusters was 90.7% and 90.6%, respectively. The data suggest that silver and bighead carps have two morphologically distinguished developmental stages.

2.3 One way analysis of variance

One way ANOVA was carried out to determine whether two clusters were statistically different in any meristic measurement. Results were shown in Tab. 9 and 10.

Eighteen indices showed highly significant difference between the two stages of silver carp. Four indices showed significant difference, while only 8 indices showed no statistical difference between the two stages. Among them, ten indices (e.g. depth/body length, snout/head length) decreased during development, while 12 indices (e. g. pectoral fin length/body length) were significantly increased during development (Tab. 9).

Fourteen indices in bighead carp showed a high significant difference between the two clusters and 3 indices were significantly different, while the remaining thirteen indices were not different. Among these, twelve indices became smaller during development, while five indices increased during development (Tab. 10)

3 Discussion

3.1 Developmental changes of silver and bighead carps

Morphological characteristics are crucial for species identification and germplasm determinations (Xie et al, 2003; Guo et al, 2004; Li et al, 2006; Qi et al, 2006; Zhao et al, 2007). However, some growth measurements were at different rates in fish, known as allometry (Osse et al, 1995). Fish body geometry changes along with development. To date, studies on geometric changes of silver and bighead carps were mainly focused on the development of hatchlings and fingerlings (Axel et al ,1990; Wan, 2004; Lin et al, 2006; Wang et al, 2008; Shan et al, 2009; Huysentruyt et al, 2009). Few studies have been done on the development these species from juvenile to adult (Lu, 2008).

Silver and bighead carp are fast growing, large freshwater species. The largest individuals can grow up to 40 kg. Parents of these two species spawn in rapid water. Fingerlings and parents actively migrate to lakes or quiet regions of river for feeding. Silver carp reaches sexual maturity at 3 years of age. Bighead carps reaches sexual maturity at 4-5 years of age. The current studies analyzed conventional and truss measurements of these species from 1 to 5 years of age. Cluster analysis of meristic measurements suggests that silver carps can be divided into three clusters: 1-year-old cluster, 2- to 3-year-old cluster and 4- to 5-year-old cluster. Bighead

Tab. 9 Indices of significantly difference between two combined clusters of Silver Carps

Character	Mean values (cm)		*P* Value	Trend of change
	Cluster Ⅰ	Cluster Ⅱ		
Total length/Body length	1.214±0.017	1.177±0.026	0.000	decrease
Snout length/Head length	0.185±0.018	0.150±0.024	0.000	decrease
Eye diameter/Head length	0.183±0.020	0.132±0.020	0.000	decrease
Body depth/Body length	0.305±0.012	0.282±0.012	0.000	decrease
Depth of head/Body length	0.247±0.010	0.238±0.080	0.000	decrease
D_{1-2}/Body length	0.254±0.016	0.233±0.090	0.000	decrease
D_{9-10}/Body length	0.208±0.014	0.195±0.010	0.000	decrease
D_{3-4}/Body length	0.108±0.014	0.103±0.011	0.025	decrease
D_{5-6}/Body length	0.126±0.008	0.122±0.008	0.025	decrease
D_{1-9}/Body length	0.491±0.016	0.485±0.013	0.025	decrease
D_{2-3}/Body length	0.291±0.015	0.305±0.010	0.000	decrease
D_{8-9}/Body length	0.229±0.016	0.243±0.014	0.000	increase
D_{2-10}/Body length	0.200±0.013	0.206±0.016	0.002	increase
D_{2-9}/Body length	0.349±0.012	0.354±0.010	0.024	increase
Pectoral fin length/Body length	0.203±0.088	0.210±0.014	0.010	increase
D_{3-8}/Body length	0.299±0.013	0.317±0.010	0.000	increase
D_{4-7}/Body length	0.258±0.016	0.273±0.013	0.000	increase
D_{2-8}/Body length	0.518±0.017	0.533±0.015	0.000	increase
D_{3-9}/Body length	0.264±0.015	0.290±0.009	0.000	increase
D_{3-7}/Body length	0.358±0.016	0.367±0.012	0.000	increase
D_{4-8}/Body length	0.219±0.012	0.240±0.010	0.000	increase
D_{6-7}/Body length	0.364±0.022	0.374±0.018	0.010	increase

Tab. 10 Indices of significantly difference between two combined clusters of Bighead Carps

Character	Mean values (cm)		Value of *P*	Trend of change
	Cluster Ⅰ	Cluster Ⅱ		
Total length/Body length	1.191±0.053	1.171±0.034	0.009	decease
Total length/Body length	0.387±0.016	0.371±0.016	0.000	decrease
Eye diameter/ Head length	0.141±0.017	0.116±0.015	0.000	decrease
Body depth/Body length	0.296±0.019	0.285±0.016	0.000	decrease
D1-2/Body length	0.294±0.016	0.285±0.017	0.002	decrease
D7-8/ Body length	0.152±0.017	0.142±0. 104	0.002	decrease
D1-10/Body length	0.335±0.016	0.322±0.016	0.000	decrease
D2-10/Body length	0.231±0.016	0. 216±0.012	0.000	decrease
D2-9/Body length	0.347±0.020	0.337±0.011	0.001	decrease
D3-8/Body length	0.301±0.020	0.293±0.011	0.004	decrease
D4-8/Body length	0.228±0.017	0.218±0.012	0.000	decrease
Depth of caudal peduncle/Body length	0.096±0.009	0.092±0.014	0.048	decrease
D4-7/Body length	0.229±0.016	0.241±0.022	0.001	increase

(to be continued)

(continued)

Character	Mean values (cm)		Value of P	Trend of change
	Cluster Ⅰ	Cluster Ⅱ		
D9-10/Body length	0.171±0.015	0. 180±0.011	0.001	increase
D3-9/Body length	0.272±0.012	0.285±0.022	0.000	increase
Head depth/Body length	0.883±0.042	0.900±0.030	0.015	increase
Depth of head/Body length	0.138±0.019	0.146±0.019	0.016	increase

can also be divided into three clusters: 1- to 2-year-old cluster, 3- to 4-year-old cluster and 5-year-old cluster. However, the latter two clusters had smaller Euclidean distance, and high discrimination error rate, which suggest silver carps have greater body geometry changes between one and two years of age, while bighead have greater body geometric changes during two to three years of age. Changes in silver carp during 3 – 4 years of age and in bighead during 4 – 5 years of age may be associated with sexual maturity.

3.2 Allometry of silver and bighead carp

Clustering and discriminant analysis reveal that allometry occurs between 1 and 2 years of age in silver carps and between 2 – 3 years of age in bighead carps. One way ANOVA results indicate ten meristic measurements, including body depth, head depth, snout, and eye diameter become significantly smaller during development between 1 year of age and 2 year of age in silver carps, while twelve measurements (e. g. caudal peduncle length; origin of caudal fin to (back of head), origin of pelvic fin, origin of anal fin) significantly increased. During bighead carp development from 2 to 3 years of age, twelve meristic measurements (e.g. body depth, head length, eye diameter) significantly decreased and five measurements (e.g. head depth, caudal peduncle length) significantly increased. In summary, among 30 analyzed measurements, 22 measurements for silver carp and 17 measurements for bighead carp showed allometric growth. Therefore, for back calculation of fish growth, allometric growth characters should be avoided. For identification of species, analysis of population differences and establishment of germplasm standards, it is necessary to sufficiently consider allometric growth in these species.

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