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# Morphometric characteristics of diploid and triploid Far Eastern catfish, *Silurus asotus*

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**Abstract:** This study identified the differences in the morphometric characteristics of the truss and classical dimensions between diploid and triploid Far Eastern catfish, *Silurus asotus*, and provided methods for sorting diploid and triploid Far Eastern catfish based on morphometric observations. The significant variables were the direct distance between the anterior edge of the lower lip and the anterior insertion of the dorsal fin (DALAD), the horizontal distance between the anterior edge of the lower lip and the anterior insertion of the ventral fin (HALAV), the direct distance between the anterior edge of the upper lip and the first nostril (DAUF), the direct distance between the anterior edge of the upper lip and the second nostril (DAUS), the interorbital width (IW), and the mandible barbel length (ManBL). The more significant variables were HALAV, DALAD, DAUF, IW, and DAUS. The most useful combination of variables for separating the two groups was DALAD, IW, and DAUF, which correctly classified 85% of the catfish as triploid or diploid, and that percentage was the maximum degree of value possible ($p<0.05$). Triploid Far Eastern catfish had a high rate of growth in the head region and body depth during the first year after hatching. Triploid Far Eastern catfish had smaller heads and shorter mandible barbels than diploid Far Eastern catfish.

**Keywords:** morphometric characteristic, diploid, triploid, Far Eastern catfish (*Silurus asotus*)

## INTRODUCTION

The Far Eastern catfish, *Silurus asotus* belonging to Siluridae, Siluriformes are distributed in Korea's all rivers, Japan, China, and Taiwan, and is an important commercial fish in Korea (Chyung 1996). The market demand for this species has gradually expanded in recent years. However, the following two major limitations may reduce the profits of the Far Eastern catfish culture. First, there is a sex-related dimorphism in the growth rate, i.e., females grow much faster than males (Kim *et al.* 2001; Seol *et al.* 2008). This leads to difficulty in effective stock management and frequently results in severe cannibalism in farms during the early stage of life. Second, precocious maturation prior to the fish reaching marketable size necessitates an extended cultivation period before sexual maturity. Upon attaining sexual maturity, these fish begin to experience reduced growth rate and decrease feeding efficiency (Choi *et al.* 1992; Yang *et al.* 2015). Therefore, the induction of triploidy offers more rapid growth and contributes due to the increased production of larger catfish.

Triploidization is a technique used to generate sterile aquatic animals by taking advantage of the incompatibility in pairing the three homologous chromosomes during meiosis I (Don and Avtalion 1986; Goo *et al.* 2015; Park 2019). This technique has also been used to enhance the productivity of several fish species because of its assumed ability to increase yield by channeling the energy for gonadal development into somatic growth (Tave 1993; Gil *et al.* 2017; Lee *et al.* 2018; Park 2019). More importantly, it generates fish that are unable to breed and contribute to the local gene pool even if they were to accidentally escape from confinement. In implementing sterility of exotic fish for a certain purpose, triploidy can serve as an effective method for reducing or eliminating the environmental risks of genetically modified organisms (Dunham and Devlin 1999).

In the Far Eastern catfish farming industry, chromosome engineering techniques have been applied using genetic and breeding methods to improve productivity. Preliminary studies on this species have addressed the temperature-dependent somatic cell division cycle ($\tau_0$), nuclear division of the egg, gonadogenesis, production of gynogenetically diploid, all-female diploid, and triploid strains (Kim *et al.* 2001; Park *et al.* 2004). In particular, the induction of triploid sterile catfish by a chromosome-engineering technique is drawing attention as a way to enhance the productivity of fish farming per unit effort in the short term (Cassani *et al.* 1990).

Triploids generally have similar, if not identical, morphological and meristic characteristics to diploids (Bonar *et al.* 1988). However, several morphological differences and abnormalities have been associated with triploid fish. A variety of deformities were reported in the triploid pejerrey, *Odontesthes bonariensis* (Strüssmann and Takashima 1993), but it is not clear whether these fish were in fact triploid or aneuploid. Changes in the scale pattern and the degree of reduction in the scale cover were observed in the triploid common carp, *Cyprinus carpio*, and were attributed to differences in allelic ratios for genes controlling these traits (Gomelsky *et al.* 1992). Flajshans *et al.* (1993) described differences in pelvic fin shapes and lengths between the triploid and diploid tench, *Tinca tinca*, and Tave (1993) observed facial deformities in the triploid bighead carp, *Hypophthalmichthys nobilis* and grass carp, *Ctenopharyngodon idella*. Probably the most conspicuous and frequently described gross morphological difference in triploid fish is the development of lower jaw deformities in the triploid Atlantic salmon, *Salmo salar* (Lee and King 1994). Although conclusive data are absent at the present, this deformity should be linked to rapid growth rate in seawater (Lee and King 1994).

Morphologic differences between species or populations of fish are understood and compared by general figures or specific anatomical shapes (Strauss and Bond 1990; Park *et al.* 2018). Morphometric characteristics of fish, unlike meristic, or countable characteristics, are measured in terms of characteristics; they can be measured in millimeters. Although understanding of the morphometric characteristics of fish is limited because they can be modified by the environment, the general figure of fish is mainly determined by genetic factors (Currens *et al.* 1989; Park *et al.* 2004, 2018). Morphometric characteristics of aquatic animal are used in three major ways: to make distinctions between sex and species and to identify confusing species such as crossbreed hybrids; to study figure modifications in groups and species; to identify and classify biotypic linkages (Park *et al.* 2004, 2008, 2018). The purpose of this study is twofold; first, to determine the differences in morphometric characteristics of truss and classical dimensions between diploid and triploid Far Eastern catfish so as to provide morphometric methods to easily estimate the condition of fish, especially in the aquaculture industry; second, to broaden our knowledge of the morphometric changes that occur during the growth of this species.

## MATERIALS AND METHODS

Triploid induction of the Far Eastern catfish, *Silurus asotus* was carried out according to the method of Kim *et al.* (2001). Five mature females (average 325 ± 30.7 g body weight (BW)) were induced to spawn using a single intraperitoneal (IP) injection of 1,000 IU of human chorionic gonadotropin (hCG, Sigma, USA) per kg BW. Sperm was obtained by cutting the surgically removed testes of three males (average 215 ± 22.9 g BW) that had been given an IP injection of hCG at 500 IU $kg^{-1}$ BW. Eggs were fertilized with sperm diluted in saline using the wet method. Five minutes after fertilization, the eggs were rinsed rapidly to remove excess sperm and were immediately subjected to cold-shock treatment (4°C) for 60 min to prevent the extrusion of the second polar body. Untreated fertilized eggs were used as diploid control.

Fish were periodically sampled and their ploidy was determined by flowcytometric assessment of the nuclear DNA content in erythrocytes or fin cells (Francescon *et al.* 2004). Diploid and triploid individuals ($n = 100$) were cultivated by the method of Kim *et al.* (2001). All fish were reared in 450-L tanks under the same hydrological con-

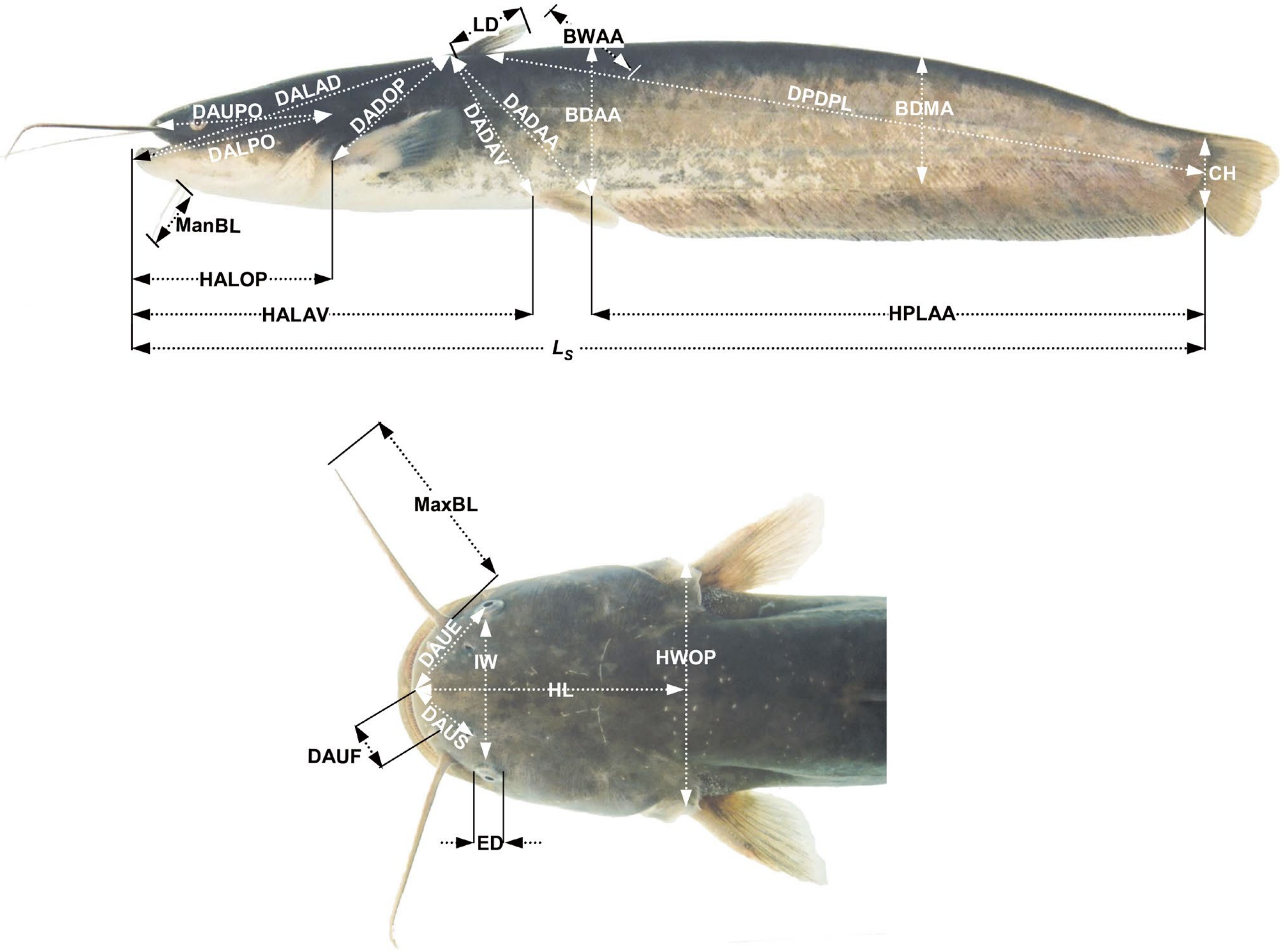

**Fig. 1.** Morphometric measurements between each landmark for diploid and triploid Far Eastern catfish, *Silurus asotus* (after Park *et al.* 2004). Top: lateral view of the whole body; Bottom: dorsal view of the head region. The measurements included standard length (*Ls*); caudal peduncle height (CH); head width between the origin of the pectoral fins (HWOP); body width at the anterior insertion of the anal fin (BWAA); maxilla barbel length (MaxBL); mandible barbel length (ManBL); head length between the anterior edge of the upper lip and the midpoint of head width (HL); eye diameter (ED); interorbital width (IW); length of the dorsal fin (LD); direct distance between the anterior edge of the lower lip and the anterior insertion of the dorsal fin (DALAD); distance between the anterior edge of the upper lip and the most posterior aspect of the operculum (DAUPO); distance between the posterior insertion of the dorsal fin and the most posterior point of the lateral line (DPDPL); distance between the most posterior point of the lateral line and the anterior insertion of the anal fin (HPLAA); distance between the anterior insertion of the dorsal fin and the origin of the pectoral fin (DADOP); distance between the anterior insertion of the dorsal fin and the anterior insertion of the ventral fin (DADAV); distance between the anterior insertion of the dorsal fin and the anterior insertion of the anal fin (DADAA); distance between the anterior edge of the upper lip and the eye (DAUE); distance between the anterior edge of the upper lip and the first nostril (DAUF); distance between the anterior edge of the upper lip and the second nostril (DAUS); the horizontal distance between the anterior edge of the lower lip and the most posterior aspect of operculum (DALPO); distance between the anterior edge of the lower lip and the origin of the pectoral fin (HALOP); distance between the anterior edge of the lower lip and the anterior insertion of the ventral fin (HALAV); the body depth at the anterior insertion of the anal fin (BDAA); and the midpoint of the anal fin base (BDMA).

ditions. Water temperature was maintained at 24±1.5°C and the mean water oxygen concentration was kept close to saturation level (mean: 9.4±0.3 mg $L^{-1}$). Experimental fish were fed twice daily, a total of 2% food of their average BW during the experimental period (3 years).

During the experiment, to avoid sampling fish with guts that were distended by large quantities of food, fish were starved for 24 hrs before sampling (Park *et al.* 2001). Samples ($n=50$, 1 year after hatching) from each group were randomly captured and anesthetized with a lidocaine-HCl/

**Table 1.** Means and standard deviations for the morphometric dimensions of diploid and triploid Far Eastern catfish, *Silurus asotus*, one year after hatching and the ANOVA results between the groups

| Morphometric dimension | 2n | 3n | ANOVA |
|---|---|---|---|
| DALAD/*Ls* | 30.32 ± 0.629 | 32.36 ± 0.783 | NS |
| DPDPL/*Ls* | 69.52 ± 3.220 | 71.70 ± 3.742 | NS |
| HPLAA/*Ls* | 55.55 ± 0.567 | 56.95 ± 2.180 | NS |
| HALAV/*Ls* | 36.90 ± 0.898 | 37.54 ± 1.168 | NS |
| HALOP/*Ls* | 19.01 ± 0.474 | 21.61 ± 0.251 | * |
| DALPO/*Ls* | 20.56 ± 0.253 | 21.64 ± 1.053 | NS |
| DAVPO/*Ls* | 19.04 ± 0.599 | 19.59 ± 0.495 | NS |
| DADOP/*Ls* | 15.50 ± 0.306 | 16.70 ± 0.404 | NS |
| DADAV/*Ls* | 17.54 ± 0.840 | 18.78 ± 1.296 | NS |
| DADAA/*Ls* | 18.75 ± 1.095 | 19.92 ± 1.296 | NS |
| MaxBL/*Ls* | 25.25 ± 1.351 | 29.52 ± 2.313 | NS |
| ManBL/*Ls* | 8.13 ± 0.679 | 8.76 ± 0.989 | NS |
| ED/*Ls* | 2.43 ± 0.114 | 2.17 ± 0.197 | * |
| LD/*Ls* | 7.64 ± 0.236 | 6.97 ± 0.448 | NS |
| CH*Ls* | 4.49 ± 0.946 | 5.80 ± 0.595 | NS |
| BDAA/*Ls* | 13.70 ± 0.300 | 16.38 ± 0.630 | * |
| BDMA/*Ls* | 11.59 ± 0.610 | 12.54 ± 0.999 | NS |
| BWAA/*Ls* | 10.06 ± 0.534 | 11.23 ± 1.249 | NS |
| HWOP/*Ls* | 15.69 ± 1.091 | 16.01 ± 0.303 | NS |
| DAUF/HL | 13.81 ± 0.784 | 14.81 ± 0.160 | NS |
| DAUS/HL | 24.97 ± 3.562 | 25.99 ± 1.121 | NS |
| IW/HL | 48.85 ± 0.725 | 49.12 ± 1.106 | NS |
| DAUE/HL | 37.08 ± 1.646 | 35.51 ± 0.685 | NS |

For dimensions (Fig. 1), refer to the text for details. Data were analyzed using student's *t*-test on data transformed to the arcsine of the square root. *$p<0.05$, NS: not significant.

NaHCO$_3$ for photographing and measuring (Fig. 1). All measurements for anesthetized individuals were taken to the nearest 0.01 cm using digital vernier calipers (CD-20CP; Mitytoyo, Kawasaki, Japan). As in study by Park *et al.* (2004), body outline measurements of samples at 3 years after hatching ($n=50$, average 34 ± 2.5 cm total length, average 187 ± 11.6 g BW) from each group were taken by 25 kinds of distances between landmarks for both truss and classical dimensions (Fig. 1). *Ls*, HALOP, HALAV, and HPLAA indicate horizontal distance, and the others indicate direct distance.

*Ls* measurements were analyzed through arcsine-square root transformation. DAUF, DAUS, IW, and DAUE were analyzed after transforming the measurements relative to head length. Student's *t*-test was used to determine the significance between diploid and triploid among the various parameters ($p<0.05$, $n=50$). Afterward, the five most significant variables were used for stepwise discriminant analysis ($n=50$) to provide the maximum separation between groups (Bonar *et al.* 1988). Differences between means were regarded as significant at $p<0.05$.

## RESULTS

At the end of the experiment, accumulated survival was 90% in the triploid Far Eastern catfish, *Silurus asotus* group, but only 75% in the diploid group. The average standard lengths (*Ls*) of the diploid and triploid groups were 21.4 ± 2.91 cm and 19.9 ± 2.48 cm, 1 year after the beginning of the experiment and 33.9 ± 2.94 cm and 38.1 ± 1.70 cm at the end of the experiment respectively.

Table 1 shows the means of the morphometric dimensions of Far Eastern catfish, 1 year after hatching and results of ANOVA testing for differences among groups. Differences between the diploid and triploid groups sig-

**Table 2.** Means and standard deviations for the morphometric dimensions of diploid and triploid Far Eastern catfish, *Silurus asotus*, three years after hatching and the ANOVA results between the groups

| Morphometric dimension | 2n | 3n | ANOVA |
|---|---|---|---|
| DALAD/*Ls* | 31.52±1.012 | 29.99±1.405 | * |
| DPDPL/*Ls* | 71.03±1.533 | 70.02±2.830 | NS |
| HPLAA/*Ls* | 60.48±2.882 | 60.94±3.217 | NS |
| HALAV/*Ls* | 37.95±1.980 | 34.64±1.609 | * |
| HALOP/*Ls* | 19.61±0.708 | 19.39±1.494 | NS |
| DALPO/*Ls* | 19.01±1.036 | 19.10±1.192 | NS |
| DAVPO/*Ls* | 17.06±0.926 | 16.68±0.711 | NS |
| DADOP/*Ls* | 15.82±2.074 | 15.01±0.996 | NS |
| DADAV/*Ls* | 17.28±1.706 | 16.51±3.068 | NS |
| DADAA/*Ls* | 20.20±1.375 | 20.45±1.872 | NS |
| MaxBL/*Ls* | 20.89±1.776 | 19.95±1.534 | NS |
| ManBL/*Ls* | 8.44±1.178 | 7.31±0.735 | * |
| ED/*Ls* | 2.07±0.214 | 1.96±0.101 | NS |
| LD/*Ls* | 6.16±1.061 | 5.74±0.511 | NS |
| CH/*Ls* | 6.28±0.721 | 7.25±0.767 | NS |
| BDAA/*Ls* | 16.73±1.047 | 16.30±1.065 | NS |
| BDMA/*Ls* | 15.07±0.938 | 15.49±0.903 | NS |
| BWAA/*Ls* | 5.89±0.569 | 7.76±0.944 | NS |
| HWOP/*Ls* | 14.92±1.356 | 14.34±0.754 | NS |
| DAUF/HL | 17.37±1.777 | 13.15±1.310 | * |
| DAUS/HL | 25.23±4.561 | 20.24±2.378 | * |
| IW/HL | 55.51±2.864 | 47.06±6.153 | * |
| DAUE/HL | 36.19±2.740 | 32.14±2.152 | NS |

For dimensions (Fig. 1), refer to the text for details. Data were analyzed using student's *t*-test on data transformed to the arcsine of the square root. *$p<0.05$, NS: not significant.

nificantly affected both the classical and truss dimensions. There were significant differences in these dimensions in the diploid group compared to the triploid group. The triploid group had drastically increased truss dimensions of BDAA/*Ls* in the trunk region and showed higher truss dimensions of BDAA/*Ls* than the diploid group. In addition, the triploid group had an increased classical dimension of HALOP/*Ls* in the head region and a higher classical dimension of HALOP/*Ls* than the diploid group. However, the diploid group had a higher ED/*Ls* than the triploid group. Significant differences between the diploid and triploid groups were not observed for the other morphometric dimensions ($p<0.05$).

Table 2 shows the means of morphometric dimensions of the fish 3 years after hatching and results of ANOVA testing for differences among groups. The same results appeared as above. Specifically, the diploid group had increased classical dimensions of DALAD/*Ls* and HALAV/*Ls* in the head region, and had higher classical dimensions of DALAD/*Ls* and HALAV/*Ls* than those of triploid group. Also, the diploid group had higher DAUF/HL, DAUS/HL, and IW/HL in the head region than the triploid group, and had higher ManBL/*Ls* in mandible barbel length than those of triploid group ($p<0.05$). However, there was no significance in any truss dimensions between the two groups.

Significant variables were DALAD, HALAV, DAUF, DAUS, IW, and ManBL (Table 3). The most useful combination of these variables for separating the two groups was DALAD, IW, and DAUF, which correctly classified 85% of the Far Eastern catfish, the maximum degree of separation obtained as triploid or diploid. Table 4 shows the classification function coefficients of the most significant variables. Classification functions (C) developed by stepwise discriminant analysis for Far Eastern catfish were for diploid catfish and for triploid catfish. Classification results of the

**Table 3.** The standardized canonical discriminant function coefficients of the most significant variables providing maximum separation between diploid and triploid Far Eastern catfish, *Silurus asotus*

| Standardized canonical discriminant function coefficients | Function 1 |
|---|---|
| HALAV | 0.232 |
| DALAD | 0.914 |
| ManBL | 0.439 |
| IW | 1.032 |
| DAUF | 2.169 |
| DAUS | 0.418 |

For dimensions (Fig. 1), refer to the text for details. Data were analyzed using student's *t*-test on data transformed to the arcsine of the square root.

**Table 4.** Classification function coefficients of the most significant variables providing maximum separation between diploid and triploid Far Eastern catfish, *Silurus asotus*

| Classification function coefficients | 2n | 3n |
|---|---|---|
| DALAD | 7.269 | 10.709 |
| IW | −6.538 | −9.262 |
| DAUF | 34.573 | 7.112 |
| (Constant) | −46.151 | −49.886 |

For dimensions (Fig. 1), refer to the text for details. Data were analyzed using student's *t*-test on data transformed to the arcsine of the square root.

**Table 5.** Classification results of the most significant variables between diploid and triploid Far Eastern catfish, *Silurus asotus*

| Ploid | Predicted group membership | | Total |
|---|---|---|---|
| | 2n | 3n | |
| 2n | 42 (85.1%) | 8 (14.9%) | 50 (100) |
| 3n | 7 (14.3%) | 43 (85.7%) | 50 (100) |

most significant variables on diploid and triploid Far Eastern catfish are seen in Table 5.

$$C = 7.269\,(\mathrm{DALAD}) - 6.538\,(\mathrm{IW}) + 34.573\,(\mathrm{DAUF}) - 46.151$$

$$C = 10.709\,(\mathrm{DALAD}) - 9.262\,(\mathrm{IW}) + 7.112\,(\mathrm{DAUF}) - 49.886$$

## DISCUSSION

The classical dimensions in which diploid and triploid 1-year-old Far Eastern catfish, *Silurus asotus* showed significant differences were HALOP/*Ls* and ED/*Ls* and those for 3-year-old Far Eastern catfish were DALAD/*Ls*, HALAV/*Ls*, DAUF/HL, and DAUS/HL. For more than 30 years, most morphometric investigations of fish have based characteristic selection on the classical dimensions of length, depth and width primarily in the head, and tail regions as described by Hubbs and Lagler (1947). These dimensions are focused on the anterior-posterior body axis and the head and caudal regions and, as a result, produce uneven and biased coverage of the body form (Li *et al.* 1993; Park *et al.* 2018).

Truss dimensions in which diploid and triploid 1-year-old Far Eastern catfish showed significant differences were HALOP and BDAA/*Ls*; that for 3-year-old Far Eastern catfish was IW/HL. The truss dimension consists of a systematically arranged set of distances measured among a set of preselected anatomical landmarks, which are points identified on the basis of local morphological features and chosen to divide the body into functional units (Strauss and Bond 1990). These dimensions, which include components of body depth and length along the longitudinal axis of the fish, have theoretical advantages over classical morphometric characteristics in discriminating among groups (Humphries *et al.* 1981; Strauss and Bookstein 1982; Winans 1984; Currens *et al.* 1989). Both truss and classical dimensions are used to describe fish body shape (Hubbs and Lagler 1947; Strauss and Bookstein 1982; Park *et al.* 2001). Currens *et al.* (1989), studying the body shapes of starved chinook salmon, *Oncorhynchus tshawytscha* and rainbow trout, *O. mykiss* using both truss and classical dimensions, pointed out that the depth of the trunk region was most affected and the caudal region least affected. Therefore, measurements of the caudal region of fish are more useful for understanding interspecific variation than are measurements of the trunk region.

Differences between diploid and triploid fish 1 year after hatching were in the head region, body depth, and eye diameter; significant variables 1 year after hatching were HALOP/*Ls*, BDAA/*Ls*, and ED/*Ls*. That is, triploid fish had longer head regions and body depths as well as smaller eye diameters than diploid fish. However, differences between diploid and triploid fish 3 years after hatching differed from those 1 year after hatching. Because the most significant variables 3 years after hatching were DALAD/*Ls*, HALAV/*Ls*, ManBL/*Ls*, DAUF/HL, DAUS/HL, and IW/HL, differences between diploid and triploid fish 3 years after hatching were in the head region, mandible barbel length, distance between upper lip and nostrils, and distance between eyes. That is, triploid fish had shorter head regions and mandible barbel lengths than diploid fish, and diploid fish had a greater distance between the upper lip and nostrils as well as between the eyes than triploid fish. The triploid fish had high growth rate of the head region and body depth during the first year after hatching. These rates then decreased and diploid fish had higher growth rate of the head region and the mandible barbel than triploid fish. Eventually, the body shape of triploid fish was characterized by a smaller head and shorter mandible barbel than diploid fish.

Bonar *et al.* (1988) made a separation of triploid and diploid grass carp, *Ctenopharyngodon idella* by external morphology, using classification functions of body depth, gape width, and cheek height. The separation rate was only 65% correct. In this study, the separation rate was 85% correct. In an attempt to further increase the accuracy of separation, although the lowest discriminant coefficient (DALAD) among the most significant variables was excluded however, this has not improved the accuracy of separation.

There are a number of techniques for separating diploid and triploid Far Eastern catfish (Kim *et al.* 2001; Gil *et al.* 2017). The three most widely accepted methods now are flowcytometric measurements of erythrocyte DNA, coulter counter estimation of erythrocyte nuclear size and measurement of erythrocyte nuclear volume by light microscopy (Thorgaard *et al.* 1982; Wolters *et al.* 1982). Unfortunately, the first two methods are very expensive and light microscopy is time-consuming and kind of inaccurate for widespread use. Clearly, any method that would be as accurate as, more rapid and less costly than these three techniques would be helpful to Far Eastern catfish culturists.

Spontaneous triploids of crucian carp, *Carassius auratus*, crucian carp, *C. cuvieri* and common carp, *Cyprinus carpio* were reported by Lim *et al.* (2015). Also, Goo *et al.* (2018) suggested that the classification of each species and classification between diploid and spontaneous triploid carp cytogenetic, hematological and histological characteristics used in their study were useful indices of ecological status in each species from the major rivers in Korea. This study has identified the morphometric characteristics that can be usefully used to separate diploid and triploid Far Eastern catfish. The results broaden our knowledge of the morphometric changes that occur in diploid and triploid Far Eastern catfish during the period of growth and should be useful for application to the aquaculture industry as well.

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