Evaluating Morphometric Character Variations in Diploid and Triploid Common Carp (*Cyprinus carpio*) During Early Developmental Stages

ABSTRACT

Numerous techniques have been put out to differentiate between diploid and triploid organisms, but they are all too complicated and expensive for everyday handling. This study investigated the possibility of distinguishing between diploid and triploid common carp by using simple and cost-effective morphometric variations. This study examines morphometric variations between diploid and triploid common carp (*Cyprinus carpio*) at three initial developmental stages at 15-, 45- and 75-days post-hatch (dph) to evaluate the morphometric variations during the early developmental stages. Assessments of 11 morphometric characteristics indicated no significant differences between both the ploidy groups at 15 and 45 dph (P >.05), implying similar growth patterns during the early stages of development. However, by 75 dph, significant differences (P<.05) were observed in standard length (SL), eye diameter (ED), anterior myotomal height (AMH), and middle myotomal height (MMH). Triploid fish exhibited higher values in these characteristics. The findings imply that the growth benefits in triploids could be related to metabolic or genetic alterations associated with triploidy, particularly as physiological differences become more pronounced over time. The similarities in early morphometric characteristics between diploids and triploids likely signify shared genetic lineages, whereas the differences at later developmental stages highlight the effect of triploidy on growth characteristics.

Keywords:*common carp, diploid, triploid, days post-hatch, morphometric variations.*

1. INTRODUCTION

The morphometric differences are considered to be based on the variations in general body shape or anatomical shape observed and are compared among populations of different species **[1].** In the context of fish, morphometric characters are the measured traits of body characters that are typically measured on a fish body like body length and width, the caudal peduncle length and width, eye diameter, etc. While the morphometric traits can be influenced by the environment, the fish body shape is completely governed by genetics factors **[2,3]**The morphometric analysis mainly concerns primarily in the following

areas of study. First, in the identification of species and gender **[4]**, also confirms species, including those of uncertain hybrid status **[5,6].** Second, the morphometric variations within populations and species can be examined **[7,8].** Lastly, it contributes to the systematic biological identification, association, and classification of organisms. For the morphometric measurements, both truss **[9]** and classical **[10]** dimensions have been used to describe fish body shape. Truss morphometric traits involve body depth and length measurements along with a longitudinal axis, it comprises of distances measured between specific anatomical landmarks which are arranged in a systematical series. These landmarks are selected based on local morphological features and are chosen to segment the body into functional units**[11].**Whereas, a fish's classical dimensions are identified by direct measurements of its body parts, which typically represent distances between specific anatomical landmarks. The dimensions include the total length, the fork length, the standard length, and the head length. Typically, they are used for identifying species, studying populations, and comparing growth patterns. Unlike truss-based morphometrics, which use interconnected measurements to form geometric networks, classical dimensions focus on linear traits that are straightforward and commonly used in fisheries biology**[3]**.As a technique in chromosome manipulation, triploidy induction has gained significant attention for its ability to produce sterile fish populations. This process of triploidy induction boosts the fish yield by transferring the energy typically used for gonadal development towards somatic growth **[12]**, it also produces fish that are incapable of reproduction, thereby preventing their interaction with the wild fish species if they escape from captivity. By inducing sterility in exotic fish for specific purposes, serves as an effective strategy for mitigating or eradicating the environmental risks associated with genetically modified organisms **[13].** Generally, the morphological and meristic traits in triploid animals may not be identical but are similar with their diploid counterparts **[14].**However, triploidy in fish have been associated with several morphological differences and abnormalities **[15].** For the rapid and accurate identification of polyploid organisms, researchers and farmers should use cost-effective and less complex technology for effective management. Morphological characterization and analysis are one such economical approach for polyploid identification and therefore this method is widely used for the recognition or determination of fish stocks **[16,17].** Analysis of phenotypic variation remains the simplest and most direct approach used to differentiate, distinguish, and categorize stocks, sexes and species of fish **[18,19, 20].**In the world,

common carp is the most important fish species for fish farming, followed by salmon, tilapia, and catfish. The sustainability of these important fish species thus requires deliberate aquaculture measure**[21]**.Common carp (*Cyprinus carpio*), is a common freshwater fish that thrives in eutrophic conditions and is found in lakes and large rivers throughout Europe and Asia **[22,23].**Eighty percent of the fish produced worldwide are common carp, which makes a substantial contribution to fish production in nations **[24].**Common Carp is the most widespread cyprinid species, contributing significantly to freshwater fish production in inland waters like lakes, reservoirs, and streams across various regions. Today, Common Carp is considered vulnerable in many of its native habitats due to a notable decrease in genetic diversity caused by the mixing of domesticated varieties with the original wild populations**[25]**.By affecting the nutrient cycle, sediment composition, and vegetation growth, common carp also referred to as engineers, as it can change the ecological characteristics of aquatic environments **[26].**However, early sexual maturity in common carp can have a substantial impact on aquaculture methods, due to this, common carp have prematurely developed reproductive organs and may grow at slower rates and have lower-quality fish overall. This fish spawns several times in a single year. As the animal's culture period extends beyond sexual development and inhibits fish reproduction, the benefits of sterility in triploid fish become more evident. This reduces the potential impact of genetic and ecological disorders associated with interactions between wild and cultured fish. A variety of morphological deformities were reported in a triploid fish, like changes in the scale pattern and the extent of scale cover reduction was observed in the triploid common carp, and were linked with differences in allelic ratios for the genes governing these features **[27]. Tave (1993) [28]**observed facial abnormalities in the triploid grass carp, *Ctenopharyngodonidella*, and bighead carp, *Hypophthalmichthys nobilis*. The development of lower jaw abnormalities in triploid Atlantic salmon, *Salmo salar*, was likely the most noticeable and commonly reported gross morphological abnormality in fish **[29].**

This study aims to enhance our understanding of the potential morphometric changes that take place during the growth of this species and identify the differences in classical morphometric dimensions between diploid and triploid common carp at their early stages. This will help in the development of morphometric methods for easily estimating fish conditions, particularly in the aquaculture industry.

2. MATERIAL AND METHODS

2.1 Ploidy induction and rearing

For this study, diploid and triploid common carp were produced at the hatchery of the Directorate of Coldwater Fisheries Research (DCFR) in Bhimtal, which is 1,370 meters above mean sea level (MSL) in the Shivalik Range of the Himalayas,located at 29° 21′ 0″ N, 79° 34′ 0.12″ E. The broodstock of common carp were reared in DCFR ponds, where breeding started in September when the water temperature ranged from 19 to 20°C. Four mature males (average 316±33.3 g body weight) and four females (average 565±34.5 g body weight) were induced to spawn using a single intraperitoneal (IP) injection of Ovatide at a dosage of .15ml/kg for males and .3ml/kg for females. The male and female fish were kept in separate tanks for a 12-hour latency period before spawning. The next day, male and female carp were kept together in identical tanks for natural fertilization, and plastic mesh bags were placed in the tanks to collect the fertilized eggs. After spawning, to retain the second polar body, the fertilized eggs in the mesh bags were transferred to a pressure chamber vessel for pressure shock induction. Ten minutes after fertilization, the eggs were subjected to a pressure shock of 6000 psi for five minutes at about 20°C. The diploid, untreated eggs were retained as control. Following the shock treatment, the eggs were incubated at an average temperature of 19.5°C in hatchery tanks. The tanks were monitored daily for mortalities, and the fish were fed twice daily with commercial feed.

2.2 Measurement of morphometric characteristics

The morphometric characteristics of diploid and triploid carp were compared at 15, 45, and 75 days after hatching. As part of this analysis, classical dimensions between diploid and triploid common carp at their early developmental stages were compared to assess potential morphometric changes during the fish's growth. A total of 40 samples were selected for the study, consisting of 20 diploids and 20 triploids. After sampling, the fish were euthanized with clove oil, and their body weight and standard length were recorded. Handling and sampling procedures complied with the guidelines of the Institute Animal Care and Use Committee of ICAR-DCFR, Bhimtal, India. Following a two-day immersion in 10% neutral

buffered formalin (NBF), the samples were washed with tap water and moved to 70% ethanol. Digital images of each fish were taken against a graph paper background using a digital camera (Canon PowerShot ELPH 180). After that, the morphometric characteristics (classical dimensions) used in the experiment were measured in these photomicrographs using Image-Pro Premier by calibrating the micrometre at 500 µm. The average weight and length of the samples, collected from 15 days post-hatch (dph), 45 dph, and 75 dph, of both diploid and triploid fish are presented in Table 1.

Eleven morphometric dimensions of both diploid and triploid common carp were measured for the study (TL, SL, HL, HD, ED, SED, AMH, MMH, PMH, LCP, BD) (Table 2, Fig 1).

Table 1:The average weight and length of the samples taken of both diploid and triploid fish at different days post-hatch (dph). Data expressed as Mean ± SD.

Days post-hatch (dph)	Diploid		Triploid	
	Average weight (g)	Average length (cm)	Average weight (g)	Average length (cm)
15	0.15±0.01	0.39±0.05	0.18±0.02	0.40±0.06
45	1.34±0.25	3.46±0.36	1.67±0.34	3.64±0.30
75	4.56±0.27	6.51±0.34	4.84±0.34	5.87±0.30

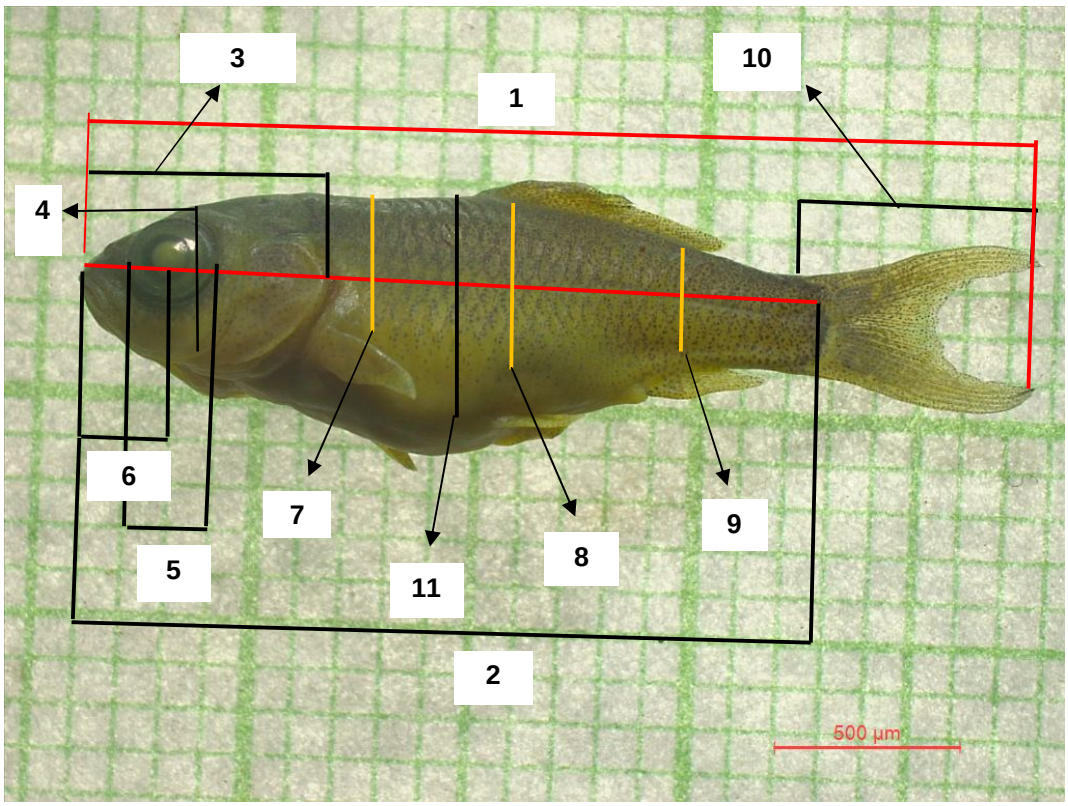

Fig 1: Morphometric dimension landmarks in common carp.

1. Total length (TL)
2. Standard length (SL)
3. Head length (HL)
4. Head depth (HD)
5. Eye diameter (ED)
6. Snout to middle of the eye (SED)
7. Anterior myotomal height (AMH)
8. Middle myotomal height (MMH)
9. Posterior myotomal height (PMH)
10. Length of caudal peduncle (LCP)
11. Body depth (BD)

Table 2: Description of the morphometric characters measured in the study

S.No.	Characters	Code	Description
1.	TL	Total length	The distance from the foremost tip of the snout to the o the posterior edge of the forked portion of the caudal fin. This measurement provides a complete evaluation of the fish's entire length and is frequently used in research on species identification, growth, and size comparisons. TL offers a basic parameter for analyzing development patterns, body form, and general health in fish and is impacted by both hereditary and environmental variables
2.	SL	Standard length	The distance from the foremost tip of the snout to the end of the caudal peduncle. SL commonly used metric that offers a more consistent basis for comparing fish of different species or developmental stages. When evaluating fish physical traits, growth rates, and body proportions, standard length is useful
3.	HL	Head length	The distance from the tip of the snout to the posterior edge of the operculum.HL is an important indicator of the size, shape, and structure of the head, all of which can differ greatly between species and developmental stages. An indicator of dietary adaptations, preferred habitats, and general growth patterns, head length is used to evaluate morphological changes between species and is impacted by both genetic and environmental variables
4.	HD	Head depth	The vertical distance, at the point of maximum depth, between the dorsal (upper) and ventral (lower) sides of the head. This measurement, which is made perpendicular to the fish's length, gives a crucial indication of the structure and form of the head, which can differ greatly between species and be impacted by both environmental and genetic variables
5.	ED	Eye diameter	The distance between the anterior and posterior edge of the eyeball.The eye size indicated by this measurement can vary greatly between species and life stages and is frequently associated with the fish's environment, feeding habits, and activity patterns (e.g., nocturnal vs. diurnal). Eye Diameter is used to evaluate adaptations to various environmental situations, such as the availability of light in the fish's natural habitat, and to compare visual capability among species

6.	SED	Snout to middle of eye	The distance between middle of the eye and the tip of the snout. In order to differentiate between species, comprehend eating adaptations, and evaluate developmental differences among fish, this measurement offers a precise indicator of snout length in relation to eye position
7.	AMH	Anterior myotomal height	The vertical height of the myotome, or muscle segment, located just behind the head and close to the anterior (front) part of the body. This measurement indicates the depth of the musculature in this area and is usually taken perpendicular to the body axis. The robustness and muscular distribution of several fish species are evaluated using AMH, which can provide information about their swimming capabilities, growth conditions and general body condition
8.	MMH	Middle myotomal height	The vertical height of the myotome, or muscle section, located around halfway along the fish's body length. The thickness of the mid-body musculature is indicated by this measurement, which is taken perpendicular to the body axis. MMH is helpful in assessing energy reserves, body condition, and muscle development, all of which can differ depending on the species growth pattern and environment
9.	PMH	Posterior myotomal height	The vertical height of the myotome, or muscle segment, located near the rear portion of the fish's body, usually right before the caudal peduncle (the narrow area of the body before the tail. This measurement, which is taken perpendicular to the body axis, provides insight into the posterior region's muscle development, which is important for propulsion and swimming efficiency. Fish species' body form, adaptations to movement, and general fitness are frequently evaluated using PMH
10.	LCP	Length of caudal peduncle	The distance between the beginning of the caudal fin (tail fin) and the posterior end of the anal fin base.The length of the slender portion of the body that joins the main body to the tail is measured here. Because differences in the caudal peduncle's length and robustness can affect a fish's ability to navigate, move quickly and propel itself, it is crucial for understanding swimming dynamics and tail movement efficiency
11.	BD	Body Depth	The distance between the origin of the dorsal fin and pelvic fin. BD

	is used to evaluate body shape which varies greatly among species and can be influenced by things like swimming patterns, habitat, and feeding strategy and indicates the overall robustness or "thickness" of the fish. Body Depth is an important metric for evaluating morphological variations among species or populations and offers information about a fish's capacity to adapt to various habitats

Source: Perdana *et al*., (2021) [30]

2.3 Statistical analysis:

To compare the results, all of the measurements were standardised by dividing by the standard length (SL). Multivariate analysis (MNOVA) was used to determine the significance of the differences in various morphometric parameters between the diploid and triploid fish ($P<.05$) followed by Tukey's multiple comparison test for determining the significant difference between the groups (IBM SPSS statistic, version 22).

3. RESULTS AND DISCUSSION

The differences in all 11 morphometric characteristics between diploid and triploid common carp at 15 dph,45 dph and 75 dph is presented in Tables3, 4 and 5 respectively and Figure 2 represents the diploid and triploid fish of 15,45 and 75 dph.

Table 3: Classical morphometric dimensions expressed as percentages of standard length of diploid and triploid common carp of 15 dph. Data expressed as Mean ± SD.

Morphometric dimensions	2n	3n
SL	15.64±0.93[a]	14.24±0.93[a]
TL/SL	1.13±0.03[a]	1.12±0.01[a]
HL/SL	0.19±0.04[a]	0.20±0.02[a]
HD/SL	0.13±0.02[a]	0.13±0.01[a]
ED/SL	0.07±0.00[a]	0.07±0.00[a]
SED/SL	0.09±0.01[a]	0.10±0.01[a]
AMH/SL	0.06±0.01[a]	0.05±0.03[a]
MMH/SL	0.08±0.01[a]	0.06±0.01[a]
PMH/SL	0.05±0.01[a]	0.04±0.00[a]
LCP/SL	0.08±0.02[a]	0.09±0.01[a]
BD/SL	0.11±0.01[a]	0.08±0.03[a]

Alphabetic subscripts (a & b) indicate significant differences between the treatments (P<0.05). TL= Totallength, SL=Standard length, HL=Head length, HD =Head depth, ED=Eye diameter, SED=Snoutto middle of the eye, AMH=Anterior myotomal height, MMH=Middle myotomal height, PMH=Posterior myotomal height, LCP=Length of caudal peduncle, BD=Body depth.

Table 4: Classical morphometric dimensions expressed as percentages of standard length of diploid and triploid common carp of 45 dph. Data expressed as Mean ± SD.

Morphometric dimensions	2n	3n
SL	20.57±2.21[a]	20.22±2.67[a]
TL/SL	1.26±0.04[a]	1.25±0.01[a]
HL/SL	0.22±0.03[a]	0.23±0.01[a]
HD/SL	0.16±0.02[a]	0.16±0.03[a]
ED/SL	0.09±0.01[a]	0.09±0.01[a]
SED/SL	0.10±0.01[ab]	0.12±0.01[a]
AMH/SL	0.08±0.02[ab]	0.10±0.01[a]
MMH/SL	0.09±0.01[ab]	0.11±0.02[a]
PMH/SL	0.07±0.02[a]	0.08±0.02[a]
LCP/SL	0.14±0.03[ab]	0.20±0.08[a]
BD/SL	0.15±0.03[ab]	0.17±0.04[a]

Alphabetic subscripts (a & b) indicate significant differences between the treatments (P<0.05).

Table 5: Classical morphometric dimensions expressed as percentages of standard length of diploid and triploid common carp of 75 dph. Data expressed as Mean ± SD.

Morphometric dimensions	2n	3n
SL	31.98±1.48[b]	35.77±5.76[a]
TL/SL	1.34±0.02[a]	1.39±0.03[a]
HL/SL	0.21±0.01[a]	0.23±0.01[a]
HD/SL	0.19±0.01[a]	0.20±0.01[a]
ED/SL	0.09±0.01[b]	0.11±0.03[a]
SED/SL	0.12±0.01[a]	0.14±0.01[a]
AMH/SL	0.12±0.04[b]	0.16±0.01[a]
MMH/SL	0.16±0.03[b]	0.20±0.04[a]
PMH/SL	0.11±0.01[a]	0.12±0.01[a]
LCP/SL	0.24±0.04[a]	0.29±0.02[a]
BD/SL	0.24±0.04[a]	0.25±0.03[a]

Alphabetic subscripts (a & b) indicate significant differences between the treatments (P<0.05).

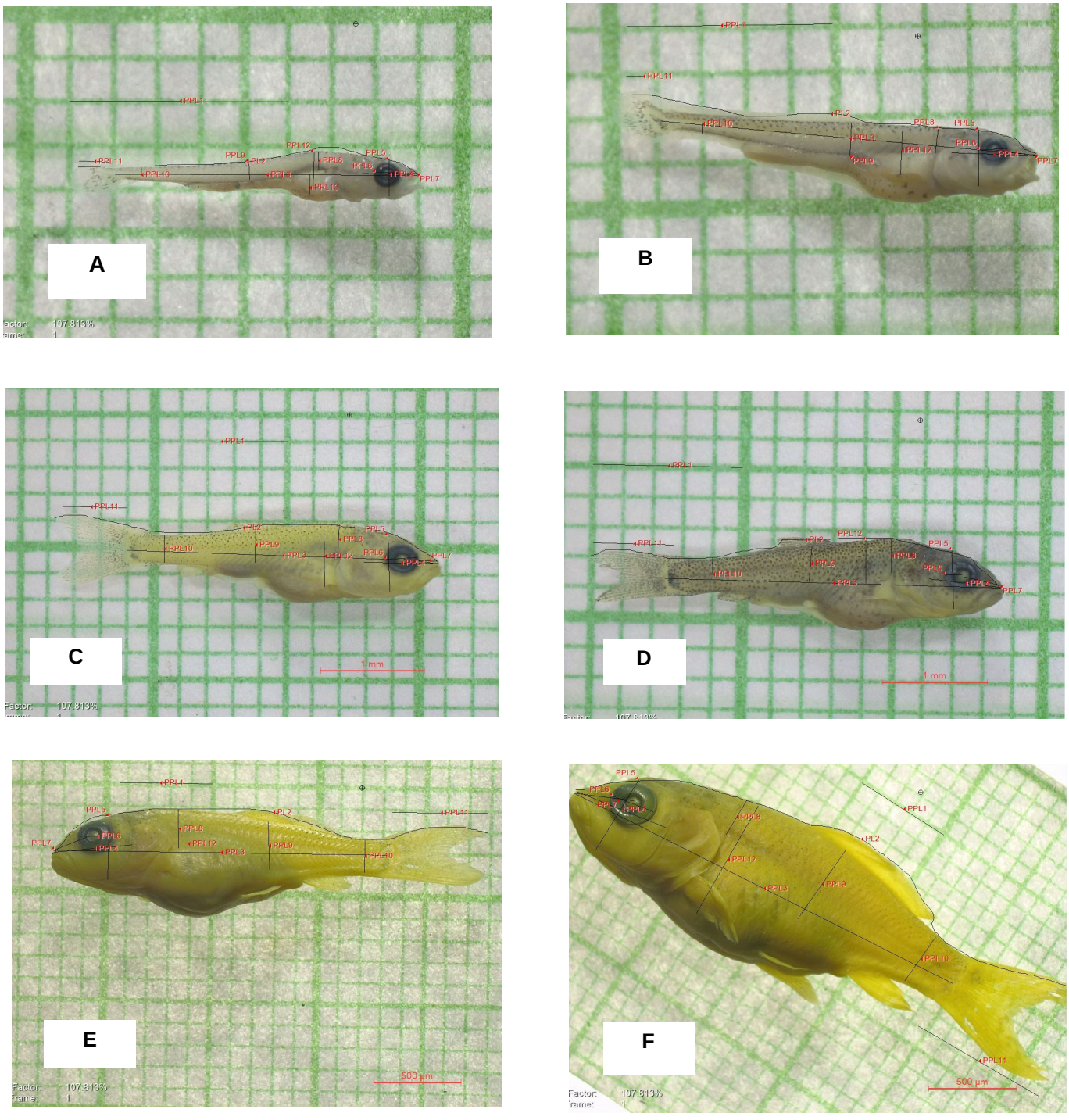

Figure 2. A=Diploid, B=Triploid (15 dph); C=Diploid, D=Triploid (45dph); E=Diploid, F= Triploid (75 dph) of common carp. Bar equals to 500 µm. (magnification 40x)

At 15dph no significant difference (*P*>.05) was seen between diploid and triploid's measured morphometric characteristics, as all the dimensions were almost equal in both the ploidy groups. Similarly, no significant difference was seen between the diploid and triploid morphometric dimensions at 45 dph. However, a significant difference was observed in some of the morphometric characteristics at 75 dph between the diploid and triploid common carp. At 75 dph the average standard length (SL) of diploid and triploid fish was 31.98±1.48 mm and 35.77±5.76 mm respectively, indicating a statistically significant difference (*P*<.05) between the groups while comparing their standard lengths. The other morphometric dimensions that showed a significant difference (*P*<.05) between the diploids and triploids included eye diameter (ED), anterior myotomal height (AMH) and middle myotomal height (MMH). The ED of the diploid was 0.09±0.01 mm. At the same time, that of triploid was 0.11±0.03 mm which aligns with a previous study, where the morphometric variation in eye diameter between the three months old diploid and triploid *Clarias gariepinus* was significantly higher (P<.05) in the triploids (0.49 ± 0.004 cm) as compared to the diploids (0.47 ± 0.005 cm) **[31]**. The AMH measured 0.12±0.04 mm in diploid fish while 0.16±0.01 mm in triploids and were significantly higher (P<.05). Lastly, the MMH of diploid was 0.16±0.03 mm while the triploids showed a higher significant value of 0.20±0.04 mm. As mentioned in a previous study that AMH and MMH are used to evaluate the growth pattern and general body condition of a fish **[30],** in the present study the AMH and MMH values were significantly higher in triploids as compared to the diploid fish, indicating that in comparison to their diploid counterparts, triploids two months post- hatch triploids exhibit better growth conditions. This also aligns with a previous study where the morphometric dimensions of spontaneous triploid common carp were significantly higher when compared with the diploid in terms of certain parameters like distance from the anterior origin of the dorsal fin to the anterior origin of anal fin (2×5), distance from the anterior origin of the dorsal fin to the anterior origin of pelvic fin (2×6), distance from the anterior origin of the dorsal fin to the origin of pectoral fin (2×7), distance from the posterior origin of the dorsal fin to the anterior origin of anal fin (3×5) and distance from the posterior origin of dorsal fin to the anterior origin of pelvic fin **[32].** Previous research examined the morphometric variations between diploid and triploid *Clarias gariepinus*, where the head length and width of the three months old post-hatch diploids and triploids were not significantly different and the values were almost equal in both the groups **[31].** Similarly, the head length and width of diploid and triploid fish at 15-, 45-

and 75-day post-hatching (dph) in the current study did not differ significantly (P > .05), with minimal variations in their values throughout the study period. **Normala*et al*., 2017[32]** reported that triploids and diploids share a common genetic background from the same parents, resulting in similar morphologies. According to previous studies, hybrids exhibit morphological differences due to the combination of chromosomes from two distinct parental sets (often from different species, genera, or families) **[33].** As a result, the offspring usually have intermediate morphological features or have a greater resemblance to one parent while retaining characteristics of the other. According to **Normala*et al*., 2017 [32]** a triploid African catfish, however, may exhibit similar morphological and genetic characteristics as its diploid counterpart due to the addition of one chromosome. This is because, meiosis produces haploid chromosomes that carry similar genetic information, which in turn gives rise to similar genetic traits in future generations. The present study found that triploid common carp exhibit similar morphological characteristics to diploid fish, particularly at 15- and 45-day post-hatch (dph). The triploid fish, however, displayed slight differences in their morphological dimensions by 75 dph, and some characteristics were significantly different from those of the diploids. Earlier in a study eight key differences between diploid and triploid grass carp fish were revealed by the measurements and counts which were body depth, predorsal length, longest anal fin ray length, suborbital breadth, cheek height, gape width and circumferential scale count as well as the number of caudal peduncles. However, no measurement among all these were 100% accurate in differentiating between diploid and triploid fish. The triploids had greater body depth, anal fin ray length, predorsal length, caudal peduncle scale counts, and circumferential scale counts than the diploids. The diploids had greater gape width, mean cheek height, and suborbital width than the triploids. Of the five most significant variables, the combination of body depth (BD), height of cheek, gape width, circumferential scale count, and caudal peduncle scale count the most effective in distinguishing the two groups was gape width (WDG) and cheek (HCK). The greatest degree of separation achieved was achieved by properly classifying 85% of triploid or diploid grass carp using these factors. This study found that triploid and diploid fish differed significantly in eight exterior traits, however the accuracy of their separation was only 65-45% **[34].** The current experiment supports the findings of this previous study of **Bonar& Thomas**, which also found that differences between diploid and triploid common carp across eleven morphometric measurements were not entirely consistent. In a

previous study an early-growth comparison of diploid and triploid rainbow trout (*Oncorhynchus mykiss*) in South Korea by **SB Lee *et al*., 2023 [35]** found that in triploid fish, no significant differences in egg-yolk morphometry or fry growth (DAH 0 to DAH 22) were observed. This means that triploid induction had a weak influence on growth at their early stage of larval development stage during the first three months after hatching (until DAH 92), diploid rainbow trout grew similarly or slightly faster than triploids, but triploids subsequently overtook diploids until the 19th week post-hatching (DAH 134), which can also be seen in the present study where no significant difference was observed in the morphometric characteristics between the diploid and triploid common carp at their early stages (15-45dph). Similarly, earlier in a study the growth of induced triploid *O. mykiss* started slower than the diploids, but accelerated thereafter in early-growth observations of 8-to-22 weeks **[36].** The morphometric differences seen at 75 days post hatch indicate that triploid common carp may start to slow growth benefits over diploids at this stage, possibly due to changes in metabolic or genetic pathways linked to triploidy. Triploids are frequently linked to increased body growth and decreased reproduction allocation, possibly clarifying their better performance in traits such as standard length, eye size, and myotomal heights. Moreover, the delayed onset of physiological changes induced by triploidy could explain the lack of significant differences observed at earlier stages (15 and 45 dph). The current research contributes to the increasing evidence that triploidy impacts particular morphometric characteristics as time progresses, enhancing growth efficiency in aquaculture settings.

4. CONCLUSION

In conclusion, this research indicates that although diploid and triploid common carp showed comparable morphometric features during the early developmental stages with no significant difference (15- and 45-days post-hatching), whereas a significant difference emerged by 75 days post-hatching. The triploid fish exhibited enhanced growth in various parameters, including standard length, eye diameter, anterior myotomal height, and middle myotomal height, implying that the benefits of triploidy become increasingly evident as the fish matures. However, relying solely on external morphological characteristics is inadequate for accurately distinguishing between diploid and triploid fish. Future investigations should aim

to improve methods for determining ploidy, such as incorporating genetic markers, to increase both accuracy and feasibility in aquaculture applications.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All applicable international, national, and/or institutional guidelines were followed (Institutional Animal Care and Use Committee of ICAR-DCFR/Ref. No. DCFR/IACUC/25/01/2021/7) for sampling, maintenance, handling and sacrificing of fish during experiments.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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