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# MORPHOMETRIC VARIATIONS BETWEEN TRIPLOID AND DIPLOID *Clarias* gariepinus (Burchell, 1822)

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ARTICLE INFO	ABSTRACT				
Received: 31 December 2016 Received in revised form: 1 July 2017 Accepted: 7 July 2017 Available online: 18 July 2017	Several scientific methods have been described in the identification of triploid fish. However, many of these methods are not applicable for routine management purposes due to their complexity and cost. In this study, the possibility of using morphological variation as a least cost and less complex method of distinguishing triploid and diploid African catfish <i>Clarias gariepinus</i> (Burchell, 1822) was examined. Triploid catfish were produced by cold shock of fertilized eggs in 5°C for 20 mins (at approximately 3 mins after fertilization). The fish were incubated, hatched and raised for 3 months. Ploidy levels of the fish were then ascertained by observing the erythrocyte shape. Triploid erythrocyte was ellipsoidal in shape while diploid was round. Morphological characterization was then carried out on				
Keywords	100 samples each of tripioid and dipioid African catfish. Although significant				
African catfish	difference between triploid and diploid African catfish could not be clearly				
Ploidy levels	distinguished. It was therefore concluded that morphological characteristics				
Chromosome number	is not ideal for discriminating triploids and diploids of African catfish. The				
Cold shock	used of erythrocyte characteristics still remains the cheapest and relatively				
Erythrocyte shape	effective method for triploid and diploid determination in African catfish.				
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#### INTRODUCTION

The African catfish *Clarias gariepinus* (Burchell, 1822) is one of the most important fish species farmed in several parts of the world. This is basically due to the high demand, better growth rate, less susceptible to disease and high survival in poor water quality (Purdom, 1972; Valenti, 1975; Fagbenro et al., 1993; Appelbaum and Kamler 2000; Adewolu et al. 2008). The production of triploid African catfish has been reported by several researchers (Henken et al., 1987; Hammed et al., 2010; Karami et al., 2010). Although, triploidization is increasingly applied in fish culture to produce sterile organisms, the geographically widespread cultivation of the African catfish, along with increasing concentrations of endocrine disrupting chemicals (EDCs) in water bodies, is continuously raising serious concerns about the reproductive potential of escaped triploid African catfish and the possibility of genetic alteration of indigenous fishes (Karami et al., 2010). Hence, the urgent need to develop management techniques for triploid African catfishes.

Generally, methods for the identification and characterization of triploids includes the use of chromosome karyotyping, flow cytometry, microfluorometry, Nucleolar Organizer Region (NOR) as well as erythrocyte measurement (Beaumont and Kelly, 1989; Manickam, 1991; Felip et al., 1997; Piferrer et al., 2000; Gheyas et al., 2001; Karami et al., 2010; Normala et al., 2016). The need to continuously sacrifice fish coupled with the continuous handling of cytotoxic chemical (colchicines) makes the routine applicability of chromosome karyotyping impossible for large scale production (Child and Watkins, 1994). More so, cost and high tech requirement for specialized equipment involved in the flow cytometry and microfluorometry make these methods also less viable for field use (Allen, 1983; Downing, 1989; Komaru et al., 1988). Erythrocyte measurement appears to be the easiest, quickest and cost-effective method (Wolters et al., 1982; Normala et al., 2016). However, several studies have demonstrated a significant overlap in the size distribution of erythrocyte between triploid and diploid samples (Gheyas et al., 2001; Peruzzi et al., 2005; Normala et al., 2016). This means the determination of triploidy based on erythrocyte measurement can be to some extent inaccurate, hence, the need to find a more suitable, rapid, adaptable and accurate method.

The use of least cost method with less complexity in terms of technology could assist farmers and researchers in rapid and accurately identification of polyploidy organism for management purposes. Morphological characterization and analysis could be one of such least cost method for polyploid identification. To date, it is still very much used in the identification of fish stocks despite technological advances in biochemical and molecular genetic (Turan 2004; Solomon et al., 2015). Analysis of phenotypic variation remains the simplest and most direct methods used to delineate, discriminate and classify stocks, sex and species of fish (Silva, 2003; Creech, 1992; Mamuris et al., 1998; Hockaday et al., 2000; Agnew, 1988 and Avise, 1994). Turan et al., (2005) and Karami et al., (2010) had earlier opined that morphological analysis is a potential tool in differentiating triploid and diploid fishes. However, till date, no study has attempted to identify polyploid fish using this method. The aim of this study is, therefore, to discriminate triploid and diploid African catfish using morphological parameters.

# MATERIALS AND METHODS

Broodstocks of African catfish (620-920 g) were collected from a local fish dealer and maintained in a one-tonne

fiberglass tank at the University Malaysia Terengganu freshwater hatchery. Fish were fed daily with commercial pellet feed (35% CP) until observation of maturity and readiness for breeding. A pair of matured male and female broodstocks was then injected with Ovaprim<sup>®</sup> at a dosage of 0.5 ml kg<sup>-1</sup> for males and 1.0 ml kg<sup>-1</sup> for females (Adebayo and Popoola, 2008). The fish were then maintained in separate tanks for a latency period of 10 h before striping was done. Eggs were collected in a clean bowl by stripping the female softly along its abdomen. Male broodstock was sacrificed to obtain the testis, and the testis lacerated to obtain the milt. The collected eggs and milt were then mixed evenly and quickly divided into separate bowls. Cold shock was applied to one of the bowls to obtain triploid African catfish while the other bowl was not cold shocked, hence, regarded as the diploid control. The cold shock protocol used was according to established baseline parameters set for this species by previous authors (Wolters et al., 1981; Richter et al., 1987; Manickam, 1991; Normala et al., 2016). This involved exposing fertilized eggs to 5°C water bath for 20 mins, at approximately 3 mins after fertilization. The eggs were incubated in triplicates batches in 100L tank (corresponding to the triploid and diploid treatment) with continuous aeration.

After hatching and egg yolk absorption, the fish were cultured for three months when they attained a weight range of 50-150g. Erythrocyte observation of the dry blood smear was done to confirm the ploidy characteristics of the fish as described by Felip et al., (1997); Felip et al., (2001) and Normala et al., (2016). Blood was obtained by cutting the caudal fin using a pair of surgical scissors without sacrificing the fish. A drop of blood was dropped on a glass slide and gently smeared using a cover slip. The smeared blood was air dried for two minutes before fixing with 95% alcohol and air dried again. The slide was then stained with 10% Giemsa stain for one hour before washing off the excess Giemsa stain with distilled water at room temperature. The Giemsastained slide was air dried, mounted with Distyrene plasticizer and Xylene (DPX) and sealed with a coverslip. A compound microscope at 40X magnification (Nikon Eclipse 80i, Japan) was used to observe the erythrocyte shape in five different blocks (Figures 1 and 2). After the status was confirmed, only was the morphological characterization attempted. Morphological parameters of 100 triploids and 100 diploid individuals were collected and analyzed. Nineteen morphometric variables were taken. They includes Standard length (SL), Predorsal distance (PDD), Pre ventral distance (PVD), Pre pelvic distance (PPD), Dorsal fin length (DFL), Anal fin length (AFL), Pectoral fin length (PFL), Pectoral spine length (PSL), Distance between occipital process and dorsal fin (DODF), caudal peduncle depth (CP), Body depth at anus

(BDA), Head length (HL), Head width (HW), Snout length (SNL), Interorbital distance (ID), Eye diameter (ED), Length of occipital fontanelle (OFL), Width of occipital fontanelle (OFW), Distance between snout and occipital processes (DSO).



Fig 1. Erythrocyte of triploid African catfish Clarias gariepinus



Fig 2. Erythrocyte of diploid African catfish Clarias gariepinus

The body related morphometric measurement was expressed as percentages of standard length while head related morphometric parameters were expressed as percentages of head length. To ensure that variations in this study were only attributed to body shape differences, and not to the relative sizes of the fish, size effects from the data set were eliminated, by standardizing the morphometric parameters using the allometric formula given by Elliott et al. (1995):

#### M <sub>adi</sub> = M (Ls / Lo) <sup>b</sup>;

Where M is the original measurement, M  $_{adj}$  is the sizeadjusted measurement, Lo is the TL of the fish, and Ls is the overall mean of the TL for all fish from all samples. Parameter b was estimated for each character from the observed data as the slope of the regression of log M on log Lo, using all fish in all groups. The morphological parameters were analyzed using Independent T-test to determine the difference between triploid and diploid African catfish. To provide an objectively defined score that summarizes the major components of variable measured between the samples, multiple group principal component analysis (PCA) of log transformed morphological variables was conducted. This allometric elimination was done using the PAST free software.

## RESULTS

Observation of the erythrocytes of the group of fish in this study showed a predominant ellipsoidal (99%) and rounded (97.5%) shape for triploid and diploid African catfish respectively (Figure 1 and 2 respectively). The morphometric parameter of cultured triploid and diploid African catfish is presented in Table 1. The result reveals significant differences in the means of fourteen out of nineteen parameters measured in this study (p < 0.05). Predorsal distance, body depth at anus, head length, head width and interorbital distance were statistically same within the treatments. Except for pre-ventral distance and pectoral fin length, higher values were recorded in triploids for the other twelve significantly different parameters compared to the diploids. However, ranges of parameters overlapped significantly even after elimination of the size effect on the data. Expressing morphological parameters as percentages of standard length (for body related parameters) and head length (for head related parameters) showed significant differences in nine out of seventeen parameters (excluding standard length and head length). Ranges of percentages were also extremely overlapping between both groups (Table 2).

Relationships of the morphometric analysis were also considered according to the 1st and 2nd Principal component (PC). PC 1 accounted for 65.05% (all positive correlations) and the PC 2 accounted for 5.24% (both positive and negative correlation) of among group variability. Together they explained 70.29% of the total among-group variability. However, plots of canonical PC 1 and 2 of the morphological data (Fig. 3 and Fig. 4) showed a broad overlap of the triploid and diploid African catfish. Analysis of the correlation matrix in the principal components shows that Predorsal distance (PDD), total length (TL), standard length (SL) and dorsal fin length (DFL) were among the characteristics most highly correlated with PC1 and all positively correlated, hence, the most influential variables for PC 1. Principal component 2 (PC2) showed both positive and negative coefficients in which width of occipital fontanelle (OFL), width of occipital fontanelle (OFW), prepectoral distance (PPD) and head length (HL) were highly correlated (Table 3).

Abbreviations	Triploid	Diploid	Significance
SL	11.73 ± 0.06° (10.02-14.38)	11.45 ± 0.06 <sup>b</sup> (10.02-14.38)	0.000*
PDD	5.99 ± 0.03 (4.16-6.83)	5.98 ± 0.05 (2.87-6.97)	0.819
PVD	5.44 ± 0.02 <sup>b</sup> (5.03-7.14)	5.52±0.03° (4.18-7.10)	0.040*
PPD	2.65 ± 0.02° (1.99-3.29)	2.58 ± 0.02 <sup>b</sup> (2.11-3.02)	0.002*
DFL	6.64 ± 0.02° (5.27-7.08)	6.42 ± 0.04 <sup>b</sup> (4.74-7.81)	0.001*
AFL	5.15 ± 0.02° (4.03-5.97)	4.96 ± 0.04 <sup>b</sup> (4.07-6.17)	0.001*
PFL	1.26 ± 0.01 <sup>b</sup> (1.04-1.49)	1.34 ± 0.01° (0.99-1.63)	0.000*
PSL	0.31 ± 0.004ª (0.20-0.40)	0.28 ± 0.005 <sup>b</sup> (0.17-0.44)	0.000*
DODF	0.83 ± 0.01 (0.66-1.15)	0.78 ± 0.01 (0.40-1.08)	0.000*
CPD	1.08 ± 0.008ª (0.76-1.28)	1.06 ± 0.009 <sup>b</sup> (0.72-1.36)	0.043*
BDA	1.74 ± 0.02 (1.28-2.40)	1.76 ± 0.02 (1.28-2.33)	0.281
HL	2.89 ± 0.01 (2.26-3.41)	2.91 ± 0.02 (1.95-3.86)	0.491
HW	1.78 ± 0.004 (1.68-1.89)	1.77 ± 0.02 (1.36-2.43)	0.863
SNL	0.92 ± 0.007° (0.55-1.13)	0.88 ± 0.008 <sup>b</sup> (0.55-1.12)	0.000*
ID	1.42 ± 0.007 (1.03-1.58)	1.42 ± 0.013 (0.99-1.90)	0.962
ED	0.49 ± 0.004ª (0.42-0.59)	0.47 ± 0.005 <sup>b</sup> (0.36-0.71)	0.015*
OFL	0.71 ± 0.005° (0.57-0.85)	0.66 ± 0.007 <sup>b</sup> (0.43-0.82)	0.000*
OFW	0.42 ± 0.005° (0.31-0.55)	0.40 ± 0.005 <sup>b</sup> (0.27-0.55)	0.001*
DSO	2.31 ± 0.02° (1.84-2.72)	2.26 ± 0.01 <sup>b</sup> (1.54-2.49)	0.008*

Table 1. Mor	phometric	parameter	of triploid	d and d	iploid (	Clarias	gariepinus

SL=Standard length, PDD=Pre dorsal distance, PVD= Pre ventral distance, PPD=Pre pelvic distance, DFL=Dorsal fin length, AFL=Anal fin length, PFL=Pectoral fin length, PSL=Pectoral spine length, DODF=Distance between occipital process and dorsal fin, CP =caudal peduncle depth, BDA=Body depth at anus, HL=Head length, HW=Head width, SNL=Snout length, ID= Inter orbital distance, ED=Eye diameter, OFL= Length of occipital fontanelle, OFW= Width of occipital fontanelle, DSO= Distance between snout and occipital processes. Note: \*P<0.05 showed significant difference between triploid and diploid Numbers are means ± standard errors (data range). Mean in the same row with different superscripts differ significantly (Independent T-test, P≦0.05, n=100). All measurements are in centimeter (cm).

Table 2.	Body a	and head	related	morphometric	parameters	expressed	as	percentages	of	standard	and	head	length	of
	triploi	d and dipl	oid Clar	ias gariepinus										

Abbreviations	Triploid	Diploid	Significance
PDD/SL	51.17 ± 0.30 <sup>b</sup> (36.22-58.13)	52.28 ± 0.43° (23.54-60.96)	0.034*
PVD/SL	46.44 ± 0.27 <sup>b</sup> (35.08-60.8)	48.25 ± 0.29° (37.41-59.54)	0.000*
PPD/SL	22.67 ± 0.17 (16.95-29.21)	22.56 ± 0.15 (18.77-26.14)	0.611
DFL/SL	56.67 ± 0.26 (44.09-60.84)	56.10 ± 0.26 (42.45-61.99)	0.128
AFL/SL	43.99 ± 0.23 (35.02-52.01)	43.32 ± 0.25 (37.93-53.94)	0.151
PFL/SL	10.78 ± 0.09° (8.88-13.50)	11.71 ± 0.10 <sup>a</sup> (8.65-14.13)	0.000*
PSL/SL	1.26 ± 0.02 (0.69-2.10)	1.24 ± 0.03 (0.70-2.21)	0.718
DODF/SL	7.13 ± 0.08° (5.46-10.32)	6.84 ± 0.09 <sup>b</sup> (3.63-9.57)	0.016*
CPD/SL	9.24 ± 0.08 (6.52-10.89)	9.23 ± 0.08 (6.49-11.19)	0.950
BDA/SL	7.18 ± 0.10 <sup>b</sup> (4.39-10.35)	7.81 ± 0.13° (4.31-13.18)	0.000*
HW/HL	61.69 ± 0.34 (53.47-78.42)	61.18 ± 0.46 (46.96-78.89)	0.405
SNL/HL	31.87 ± 0.30° (18.92-43.65)	30.28 ± 0.29 <sup>b</sup> (22.82-44.09)	0.000*
ID/HL	49.27 ± 0.32 (34.36-64.13)	49.03 ± 0.45 (35.57-64.25)	0.657
ED/HL	16.92 ± 0.17 <sup>a</sup> (13.67-23.62)	16.34 ± 0.17 <sup>b</sup> (12.17-23.47)	0.015
OFL/HL	24.46 ± 0.23° (18.91-33.33)	22.91 ± 0.27 <sup>b</sup> (14.66-33.64)	0.000*
OFW/HL	14.69 ± 0.18° (10.51-22.74)	13.82 ± 0.18 <sup>b</sup> (9.39-18.47)	0.001*
DSO/HL	79.93 ± 0.60° (62.38-113.55)	77.78 ± 0.56 <sup>b</sup> (58.64-106.99)	0.009*
BDA/HL	7.18 ± 0.10 <sup>b</sup> (4.39-10.35)	7.81 ± 0.13° (4.31-13.18)	0.000*

SL=Standard length, PDD=Pre dorsal distance, PVD= Pre ventral distance, PPD=Pre pelvic distance, DFL=Dorsal fin length, AFL=Anal fin length, PFL=Pectoral fin length, PSL=Pectoral spine length, DODF=Distance between occipital process and dorsal fin, CP =caudal peduncle depth, BDA=Body depth at anus, HW=Head width, SNL=Snout length, ID= Inter orbital distance, ED=Eye diameter, OFL= Length of occipital fontanelle, OFW= Width of occipital fontanelle, DSO= Distance between snout and occipital processes. Note: \*P<0.05 showed significant difference between triploid and diploid. Numbers are means ± standard errors (data range). Mean in the same row with different superscripts differ significantly (Independent T-test, P≦0.05, n=100).



PC 1 (65.05 % of variance)

- **Fig 3.** Principal component analysis of transformed morphometric data taken from triploid samples (*n*=100) and diploid fish samples (*n*=100)
- The biplot shows individual fish scores for PC 1 (65.05 % of variance) vs. PC 2 (5.24 % of variance). O= diploid; + = triploid.





**Fig 4.** Varimax factor scatter plot of transformed morphometric data taken from triploid samples (*n*=100) and diploid fish samples (*n*=100)

The scatter plot shows individual fish scores for factor 1 (98.61 % of variance) vs. factor 2 (0.77 % of variance). O= diploid; + = triploid.

#### DISCUSSION

Ploidy levels of the African catfish were easily ascertained by mere observation of the erythrocytes shape. Ellipsoidal and rounded erythrocytes were the specific characteristics of the triploid and diploid African catfish respectively. Normala et al. (2016) had opined that the observable shape

Table	3.	Principal	com	ponent	analy	analysis		trans	formed	l
		morphom	etric	charac	teristic	of	tri	biolc	African	I.
		catfish (n=	100)	and dip	loid Af	rica	n ca	atfish	( <i>n</i> =100)	)

Variable	PC 1	PC 2
Standard length	0.25	-0.03
Total length	0.25	-0.07
Predorsal distance	0.25	-0.07
Pre anal distance	0.23	-0.06
Preventral distance	0.24	-0.14
Prepectoral distance	0.23	-0.02
Dorsal fin length	0.25	-0.04
Anal fin length	0.24	-0.03
Pectoral fin length	0.17	-0.02
Pectoral spine length	0.20	0.22
Distance between dorsal and caudal fin	0.14	-0.19
Distance between occipital process and dorsal fin	0.17	0.20
Caudal peduncle depth	0.21	-0.06
Body depth at anus	0.22	-0.14
Head length	0.23	-0.16
Head width	0.22	-0.05
Snout length	0.20	-0.07
Inter orbital distance	0.22	-0.12
Eye diameter	0.19	0.22
Length of occipital fontanelle	0.14	0.61
Width of occipital fontanelle	0.13	0.59
Distance between snout and occipital processes	0.23	-0.05
Eigen value	14.31	1.15
% of variance	65.05	5.24
Cumulative % variance	65.05	70.29

Eigenvalue is more than 1 was selected (Kaiser, 1961). The first three principal components accounted for 70.29 % of the variance. Value in the body of the table are component loading.

differences between triploid and diploid are largely because of the increment of one chromosome set in the triploid fish. Benfey (1999) had earlier also postulated that the change in cell shape, as well as increase in cell size, is possibly a cytoplasmatic adjustment to the increase in nuclear size, hence, causing an enlargement of the cellular major axis more than the cellular minor axis. A similar observation has been reported for the erythrocyte of triploid Wels catfish *Silurus glanis* Linnaeus, 1758 (Flajšhans, 1997), Pond loach Misgurnus anguillicaudatus (Cantor, 1842) (Gao et al., 2007), Caspian trout Salmo caspius Kessler, 1877 (Dorafshan et al., 2008), Nile tilapia Oreochromis niloticus (Linnaeus, 1758) (Pradeep et al., 2011). Despite statistically significant differences observed in many morphological parameters measured, there was, however, no specific range or value that could be used to differentiate between triploid and diploid African catfish due to wide overlap. Differences in measurement could be said to be relative and not absolute between the two sets. A similar conclusion was made by Marques et al. (2006) when they observed significant differences in morphological parameters but low differentiation as a result of the high overlap of individuals in flatfish sample of different populations. The wide overlap of morphometric parameters as observed in both PC1 and PC2 further justifies the assumption made for this study, hence, both triploid and diploid populations were hardly discriminated from one another. To further observe clearly which morphometric characters could be used to discriminate both groups in this study, the contributions of morphometric variables to the principal component (PC) were examined. Principle component with eigenvalue more than one were selected for further analyses based on Kaiser (1961). Jolicoeur and Mosimann (1960) had earlier postulated that any component having all coefficients of the same sign was indicative of size variation, whereas that with both positive and negative coefficients was indicative of shape variation. Therefore, it can be concluded that PC1 in this study did illustrate size variation while PC2 showed shape variation between triploid and diploid fish. However, analysis of the correlation matrix revealed a mixed number of correlations with no clear pattern emerging, hence, no discrimination could be made as a result of the high overlap of individuals between the two sets of samples. Similar observation of complete overlap in the meristic count of wild and cultured C. gariepinus (Solomon et al., 2015) and Nile Tilapia Oreochromis niloticus (Olufeagba et al., 2015) has been opined as evidence of low or no variability. However, contrary to the present study, the different groups in Solomon et al. (2015) and Olufeagba et al. (2015) were easily discriminated using morphological parameters. It is well known that morphometric characters show high plasticity in response to differences in environmental conditions, such as food abundance, temperature, habitat differences etc. (Allendorf and Phelps, 1988; Swain et al., 1991; Wimberger, 1992; Olufeagba et al., 2015 and Solomon et al., 2015). Phenotypic characteristics are, however, largely determined by genotype (Rothwell, 1993) or the interaction between the genes and the environment (Soares et al., 1999). The used of broodstocks of common breeding history for the production of triploid and diploid samples couple with the rearing of these experimental fish under the same environmental and experimental conditions would have nullified and avoided possible genotype-environment interaction effect. Hence, this led to the expression of similar phenotype character possibly dictated by similar genetic information inherited from the same gene pool. The findings of this study suggest that increasing the number of chromosomes possibly do not have a fundamental effect on the external morphology of the fish. However, several previous studies have shown a significant effect of increased ploidy levels on some physiological characteristic of many fishes (Wolters et al., 1982; Sugama et al., 1992; Siraj et al., 1993; Felip et al., 1999; Cal et al., 2006). Hence, from the findings of this study, it could be right to say that morphometric analysis may not be ideal in differentiating ploidy levels of African catfish.

To our knowledge, comparative study of the morphological parameters of triploid and diploid from any species has not been reported. However, many studies exist on the morphological comparison of triploid and diploid hybrids gotten from distant hybridization between different species, family, and genus of fish. The changes in chromosome structure such as in hybrid fishes have been reported to influence morphological characteristic in fish (Masser and Dunham 1998). The study of Masser and Dunham (1998) showed that hybrid of the Channel catfish Ictalurus punctatus (Rafinesque, 1818) × Blue catfish Ictalurus furcatus (Valenciennes, 1840) resulted in distinct morphotypes as a result of increment of chromosome of the hybrids. The result of Na-Nakorn et al. (1993), on the chromosome characteristics of hybrids from the cross between male Striped catfish Pangasianodon hypophthalmus (Sauvage, 1878) and female Bighead catfish Clarias macrocephalus Gunther, 1864 also demonstrated the presence of diploid, triploid and aneuploidy hybrids corresponding respectively to two intermediate morphotypes (pangasiid-like and clarrid-like) and one morphotype indistinguishable from its clariid parent. Morphological differences in hybrids reported in these literatures are possible due to chromosome combination from two different sets of parental chromosomes (different species, genus, and family). Hence, it resulted in intermediate morphological feature or close resemblance with one of the combination but with the feature of the others. In the current study, this situation is different because the increment of one chromosome number in triploid African catfish possibly had similar morphological as well as genetic characteristics as the diploid fish. This is because during meiosis, haploids chromosome is produced which have equivalent genetic information, hence, contributing similar genetic information to the next generation (Anon, 2010). It is clear that the genetic makeup of the triploid and diploid fish is common from the same parents thereby resulting in similar morphological characters.

# CONCLUSION

In general, it can be concluded that no discrimination can be made using external morphological variables of triploid and diploid African catfish; hence, this method may not be ideal to clearly distinguish these groups of fish. The used of erythrocyte measurement still remains the cheapest, more suitable and relatively accurate method for the determination of triploid and diploid African catfish. Future study can investigate the use of genetic markers in ploidy determination.

### Sažetak

# MORFOMETRIJSKE VARIJACIJE IZMEĐU TRIPLOIDA I DIPLOIDA *Clarias gariepinus* (Burchell, 1822)

Nekoliko znanstvenih metoda ranije je opisano za prepoznavanje triploidnih riba. Međutim, mnoge od tih metoda nisu primjenjive u svrhu rutinskog upravljanja radi njihove složenosti i troškova. U ovom radu istraživana je mogućnost korištenja morfoloških varijacija kao jeftine i jednostavne metode razlikovanja triploidnih i diploidnih afričkih somova Clarias gariepinus (Burchell, 1822). Triploidski somovi su proizvedeni metodom hladnog šoka oplođenih jaja na 5°C tijekom 20 minuta (približno 3 min. nakon oplodnje). Ribe su bile inkubirane, izvaljene i uzgajane tijekom 3 mjeseca. Plodnost riba utvrđena je promatranjem oblika eritrocita. Triploidni eritrocit bio je elipsoidnog oblika dok je diploidi bio okrugli. Morfološka karakterizacija je provedena na 100 uzoraka triploidnih i na 100 uzoraka diploidnih afričkih somova. lako su značajne razlike zabilježene u mnogim parametrima, glavna morfometrijska razlika između triploidnih i diploidnih afričkih somova nije se mogla jasno razlikovati. Stoga je zaključeno da morfološka svojstva nisu idealna za razlikovanje triploida i diploida afričkog soma. Upotreba eritrocitnih karakteristika i dalje je najjeftiniji i relativno učinkovit način za triploido i diploidno određivanje afričkih somova.

Ključne riječi: afrički som, stupanj ploidnosti, broj kromosoma, hladni šok, oblik eritrocita

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