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Biometric analysis on three offspring of transgenic mutiara catfish (*Clarias gariepinus*)

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Abstract
This research was conducted at the hatchery and the Laboratory of Biotechnology of the Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran from October to December 2021, with the aim of detecting deformities and phenotypic character abnormalities in G_1, G_2, and G_3 transgenic mutiara catfish (*C. gariepinus*). The study used a completely randomized design with four treatments (G_1, G_2, G_3 as transgenic mutiara catfish, and non-transgenic mutiara catfish as controls) with three replications. Each treatment used 10 fish (test sample). The results showed the phenotypic characters of body weight (BW), total length (TL), standard length (SL), head length (HL), head width (HW), dorsal length (DL), pectoral length (PCL), and caudal length. (CL) inheritance in G_1, G_2 and G_3 was asymmetric (SD > 1.0). All biometric characters in growth control fish were symmetrical, indicated by SD values < 1.0. Qualitative biometric analysis showed that abnormal fish (body defects) were only found in two fish in G_2 and one fish in G_3 from a total sample of 10 fish. However, 1 non-transgenic (control) fish was also found. Overall, these defects were only found on the pectoral fins, both in transgenic and non-transgenic catfish, and had little effect on fish growth (still normal).

Keywords: Abnormality, deformity, transgenic mutiara strain, biometrics

1. Introduction
The African catfish species is a catfish species that has great potential as a fishery commodity, but due to lack of proper broodstock management, its genetic quality has decreased, marked by a decrease in growth rate and morphological irregularities (defects). Several breeds of African catfish strains developed in Indonesia (paiton, sangkuriang, dumbo and Egypt), does not show growth performance as desired by fish farmers due to decreased growth and increased deformity inherited in offspring [1, 2].

To improve the genetic performance of catfish distribution in the fish farmers, the Sukamandi Fish Breeding Research Institute, West Java, Indonesia has produced the mutiara strain of catfish as a result of catfish breeding activities [3]. However, after several generations, growth stability decreased, and was characterized by an increase in pectoral fin deformity by 14% in both mutiara, dumbo, sangkuriang and paiton strains of catfish. In marine fish, especially snapper, yellowfin bream (*Acanthopagrus australis*) has a dorsal fin deformity rate of about 10% [2]. The genetic improvement of mutiara catfish growth has been carried out by inserting the dumbo catfish growth hormone gene, *CgGH* (*Clarias gariepinus Growth Hormone*, 600 bp) through transgenesis [4, 5, 6, 7] to maintain the stability of fish growth. In G_1, G_2 and G_3 transgenic mutiara catfish, the level of morphological abnormalities is not known.

Abnormalities in offspring are common in living things and are associated with fish phenotypic characters such as abnormal fish body shape, albino color, fin defects [2, 8]. The effect of the abnormality causes deformity of body shape, but fish can still lay eggs with fecundity and larval survival below normal fish. Detection of abnormalities with a biometric approach is an evaluation of the resulting generation. Crosses of brood fish that have relatively close kinship tend to increase the abnormality. The quantitative and qualitative morphometric data base on three generations of transgenic mutiara catfish analyzed was the aim of this study.

2. Materials and Methods

2.1 Test fish samples and biometric analysis

The number of the G_1, G_2, G_3 transgenic mutiara catfish and G_1 non-transgenic mutiara (adult stage) was taken as many as 10 fish for each treatment from each fish rearing tank (30 fish stock/tank) for biometric character measurement. Identification of transgenic mutiara catfish using PCR assay with GH-F primers (5'-ATGGCTCGAGTTTTGGTGCTGCT-3') and GH-R primers (5'CTACAGAGTGCAGTTGGAATCCAGGG-3') [6, 9].

2.2 of the G1, G2, G3 transgenic mutiara catfish

Screening for identification of the G_1, G_2, G_3 mutiara catfish using small pieces of 10 different fish tail fins for each treatment as template DNA for PCR testing following My Taq™ HS-Red mix kit instructions (Bioline, London, UK). PCR programme settings as follows: 95 °C for 3 min; 40 cycles of 95 °C for 15 s, 55 ºC for 30 s, 72 °C for 1 min and 72 °C for 3 min. Transgenic positive catfish were indicated by the presence of a 600 bp band [9].

2.3 Biometric Analysis

The biometric characters measured for quantitative abnormality analysis included BW, TL, BD, ED, SL, HL, HW, OPL, DL, AL, PCL, CL and PVL. Meanwhile, the qualitative biometric analysis includes head shape, completeness of fins and the fish head shape. Quantitative data processing used one-way ANOVA and Duncan's multiple range test for differences between treatments at the 5% level, on the other hand, qualitative data were analyzed in a comparative descriptive.

The number of fish abnormalities in each treatment was calculated using the formulae:

$$AB = \frac{\Sigma Ia}{\Sigma Ti} \times 100\%$$

AB = abnormality
∑Ia = number of fish abnormal
∑Ti = total number of fish

3. Results and Discussion

3.1 Verification of transgenic catfish

The results of PCR amplification for screening G_1, G_2, G_3 positive transgenic mutiara catfish were indicated by the presence of a 600 bp DNA band (the *CgGH* gene) as insertion of the African catfish growth hormone gene in the transgenic Mutiara catfish genome. The results of the amplification of the PCR product of fish fin samples, showed that samples G_1, G_2, G_3 were positive for transgenics (Figure 1).

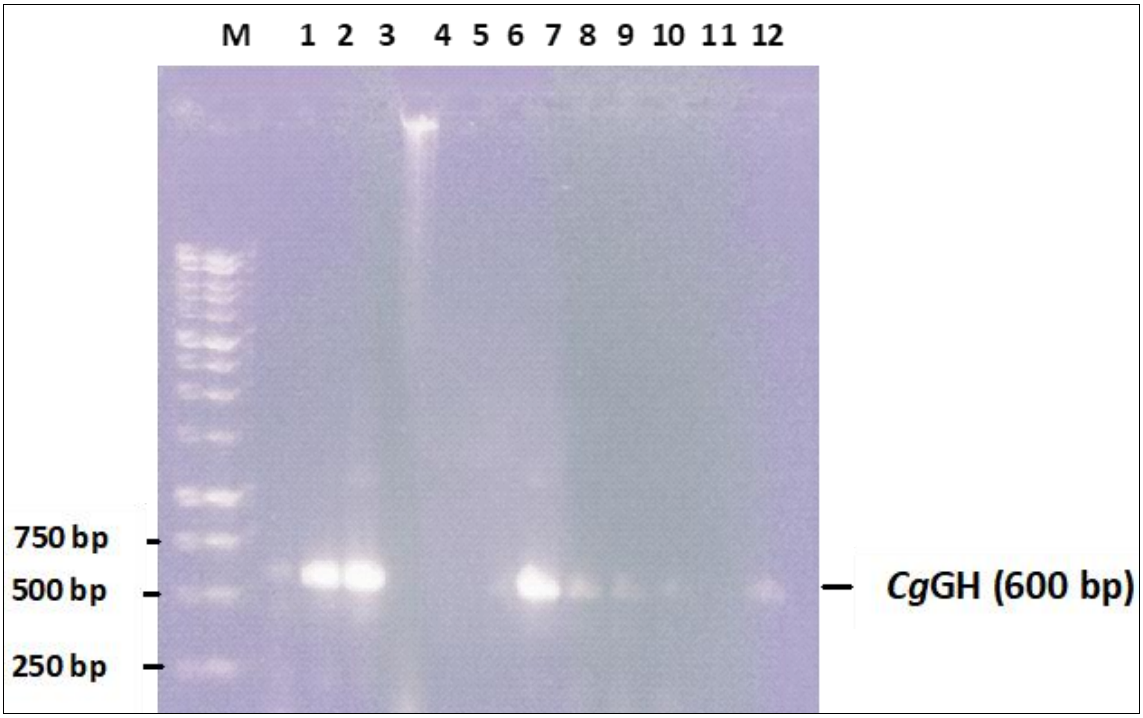

Fig 1: Confirmation of transgenic mutiara catfish; M: 1 kb DNA ladder; 1-3: sample G1 (fish number 1,2,3); 4-5: non-trasgenic samples (fish number 1,2); 6-8: sample G2 (fish number 1,2,3); 9-10: sample G3 (fish number 1,2); 11: non-transgenic sample (fish number 3); 12: sample G3 (fish number 3)

The results of the confirmation of the PCR test above (Figure 1), showed that the transgenic mutiara catfish samples were G1 (Figure 1 1st well, 2nd, 3th), G_2 (Figure 1 6th well, 7th, 8th well), G_3 (Figure 1 9th well, 10th, 12th) are transgenic fish. This was indicated by the *CgGH* fragment (600 bp size) for G_1, G_2, G_3 transgenic mutiara catfish and for non-transgenic mutiara catfish that did not contain *CgGH*.

3.2 Biometric characters

The results of biometric measurements of BD, ED, SL, HL, HW, OPL, DL, AL, PCL, CL, PVL, TL, and BW transgenic mutiara catfish (G_1, G_2, G_3) (Table 1), quantitatively showed growth characteristics higher than non-transgenic fish (SD value is higher than non-transgenic). The growth of this quantitative phenotype is classified as asymmetric.

Table 1: Biometric means of transgenic and non-transgenic mutiara catfish

Parameter	Transgenic fish			Non-transgenic (Control)
	G_1	G_2	G_3	
BW (kg)	2.96 ± 0.31[a]	1.53 ± 0.24[b]	1.75 ± 0.20[b]	0.96 ± 0.10[c]
TL (cm)	74.94 ± 3.91[a]	52.75 ± 8.61[b]	62.38 ± 2.41[c]	52.00 ± 0.76[b]
BD (cm)	5.80 ± 0.08[a]	5.83 ± 0.60[a]	5.75 ± 0.72[a]	3.77 ± 0.09[b]
ED (cm)	0.52 ± 0.02[a]	0.50 ± 0.05[ab]	0.41 ± 0.05[b]	0.57 ± 0.03[a]
SL (cm)	47.87 ± 1.16[a]	44.17 ± 2.88[a]	40.11 ± 5.76[b]	46.16 ± 0.92[a]
HL (cm)	13.18 ± 0.60[a]	9.73 ± 0.65[b]	8.82 ± 1.61[b]	9.94 ± 0.34[b]
HW (cm)	8.83 ± 0.23[a]	7.96 ± 0.77[a]	6.86 ± 1.19[b]	8.11 ± 0.25[a]
OPL (cm)	6.54 ± 0.03[a]	7.23±0.49[a]	6.48 ± 1.03[a]	7.61 ± 0.19[a]
DL (cm)	32.81 ± 0.42[a]	28.90 ± 1.71[b]	25.85 ± 3.19[b]	31.07 ± 0.78[a]
AL (cm)	19.88 ± 0.57[a]	17.26 ± 0.97[b]	17.26 ± 0.97[b]	19.11 ± 0.25[a]
PCL (cm)	6.12 ± 0.25[a]	4.44 ± 1.28[b]	3.88 ± 1.25[b]	5.66 ± 0.28[a]
CL (cm)	7.46 ± 0.03[a]	5.55 ± 0.70[b]	4.67 ± 0.99[b]	5.83 ± 0.16[b]
PVL (cm)	4.26 ± 0.33[a]	4.03 ± 0.20[a]	3.28 ± 0.70[b]	4.50 ± 0.16[a]

Data are represented as means ± SEM. Across rows, means followed by the same letter are not significantly different ($p \geq 0.05$).

Standardization of asymmetrical and symmetrical growth, SD values can be shown. SD values 0-1.0 are categorized as symmetrical growth and SD values 1.0 indicate asymmetric growth[10,11]. The biometric characters of TL, SL, HL, HW, DL, and PCL in G_1, G_2, G_3 transgenic mutiara catfish (Table 2) have asymmetrical growth (SD > 1.0) as a phenotypic feature of the transgenic fish. SD values of biometric characters (BW, BD, SL, HL, OPL, DL, HW, AL, PCL, and PVL) G1 transgenic mutiara catfish were higher than non-transgenic fish as an implication of the effect of exogenous hormone insertion [12]. Meanwhile, the biometric characters of BD, ED, OPL, AL and PVL in G1, G2, G3 transgenic mutiara catfish showed symmetrical growth (SD < 1.0), where the SD values between generations were not much different, indicating that the inheritance of these phenotypic characters was normal (SD < 1.0). All biometric characters in growth control fish were symmetrical, indicated by SD < 1.0.

3.3 Abnormality

Morphologically, G_1, G_2, G_3 and non-transgenic mutiara catfish showed normal biometric characters like normal catfish (Table 1). However, there were fish with deformities (body defects) found in G_2 and G_3 transgenic mutiara catfish as many as two and one fish, respectively from of a total of 10 fish. In addition, one non-transgenic mutiara catfish (control) were found to have abnormalities (Table 2). Generally this body defect is found only on the pectoral fins, both in transgenic and non-transgenic catfish, and has little effect on fish growth.

Table 2: Abnormalities in transgenic and non-transgenic mutiara catfish

Generation	Number fish		Abnormal (%)
	Complete fins	Incomplete fins	
G1	10	0	0
G2	8	2	5
G3	9	1	2.5
Control	9	1	2.5

The G_1 transgenic mutiara catfish had an abnormality value of 0% (Table 2). This indicates that the growth of transgenic mutiara catfish is normal for all measured phenotypic characters (Table 1). The growth abnormality of all phenotypic characters in G1 transgenic mutiara catfish of 0% indicates that the growth of the anatomical parts of the fish is symmetrical or the growth is normal. Meanwhile, G_2 transgenic mutiara catfish has an abnormality value of 5%. This indicates that in the second generation of transgenic mutiara catfish, only two fish with pectoral fin deformity (PCL) were found (Table 2). Meanwhile, in the control, one abnormal fish was found on the pectoral fins, which indicates that the level of abnormality in G_2 transgenic mutiara catfish is relatively small. For comparison, it can be shown that the results of the study of pectoral fin abnormalities in non-transgenic mutiara catfish were higher (2-14%) than in G_2 transgenic mutiara catfish (5%) [3]. This indication shows that the PCL phenotypic character abnormality in G_2 transgenic mutiara catfish is low.

G3 transgenic mutiara catfish had an abnormal value of 2.5% for PCL phenotypic characters (Table 2). The PCL phenotypic abnormality in G_3 fish is classified as low, when compared with the overall abnormality rate in non-transgenic mutiara catfish of 4%[3] and the dorsal fin abnormality level of yellowfin bream (*A. australis*) of 10%[2]. The level of pectoral fin abnormality in the G_3 transgenic mutiara catfish was also the same as the PCL phenotypic abnormality in the control of 2.5%, indicating that the effect of transgenesis did not significantly affect the normal growth of the pectoral fin in G_3 transgenic mutiara catfish.

Especially for the head shape abnormality of G_1, G_2, G_3 transgenic mutiara catfish, it did not show acromegaly head shape as shown in zebrafish and coho salmon (*Onchorhynchus kisutch*) [13, 14]. This growth remains normal or symmetrical and does not affect the overall biometric growth of fish. These indications suggest that *CgGH* does not induce the growth of acromegaly.

4. Conclusions

The biometric characters of TL, SL, HL, HW, DL, and PCL were asymmetric and the BD, ED, OPL, AL, PVL phenotypes of G1, G2, G_3 transgenic mutiara catfish were symmetrical. The G_1, G2 and G_3 transgenic mutiara catfish abnormality was relatively small (2.5-5%) generally found only on the pectoral fin, and *CgGH* insertion did not induce acromegaly.

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6. Authors' contributions

IDB and II take samples in the hatchery and collect research data. IDB, YM and EPL compiles research articles and biometric analysis and article submissions. Author also critically reviewed the manuscript for final approval to be published.

7. Competing interests

The authors declare no competing interest.

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