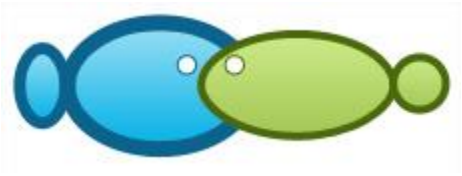

Reproduction, growth, fillet proportion and proximate analysis of tetraploid X diploid derived striped catfish (*Pangasianodon hypophthalmus*) triploid

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Abstract. Induction of triploid striped catfish (*Pangasianodon hypophthalmus*) through physical shock to obtain a high-quality seed has been done, but the physical shock reduces hatching rates and limits the number of offspring. Cross-breeding of tetraploid with diploid striped catfish is expected to be a solution in overcoming these problems. This trial of non-shocked triploid seed mass production was the first test conducted in Indonesia. The treatments were crossing between 4n♂ x 2n♀ and 2n♂ x 4n♀ striped catfish with one control. The results showed that the treatment of 4n♂ x 2n♀ produced a better hatching rate of about 19.76% but still lower than the control diploid. The determination of the ploidy level from the progenies was done by observing the maximum number of nucleoli, chromosomes counting and GH-Actin ratio with the results being 100% triploid. At nursery, for 2 months of rearing, the growth of diploid and triploid juveniles was the same. The histology indicated that the gonad of male and female triploid does not develop. This causes the yield of fillets to have a significant percentage, with the difference in meat weight up to 200 g. The meat color and proximate composition did not show a statistical difference.
Key Words: crossbreeding, feeding, performance, ploidy determination, survival.

Introduction. Innovations and breakthroughs continue to be made to improve aquaculture performance. Approved performance can be related to the optimization of feed utilization (Da et al 2016; Tiamiyu et al 2016; Jayant et al 2017), resistance to pathogens (Levy-Pereira et al 2018; Castillo et al 2019), adaptation to environmental fluctuations (Soyano & Mushirobira 2018), manipulation of hormonal reproduction (Sherwood 1987), modern and conventional genetic engineering (Arai 2001) and combination of various disciplines of study (Thorgaard et al 1992; Antunes 2015).

Striped catfish (*Pangasianodon hypophthalmus*) is a superior aquaculture commodity in Southeast Asia that has an export market in several European and American countries. It is marketed essentially as filet. In Indonesia, *P. hypophthalmus* is marketed in the form of fresh fish, smoked fish and bloater. The culture of *P. hypophthalmus* has aș constraint the form. A long time is required to reach the standard size of filleting, which is 1-2 kg per head (Akter et al 2014) would take up to one year of growth. Other constraints are the harvest size fluctuations of 12-20% per production cycle and a high feed conversion, which is 1.5. The approach to solving this problem is doing by producing triploid *P. hypophthalmus* because it has been proven to increase growth by up to 40% more than diploid *P. hypophthalmus* (Ibrahim et al 2017). However, it is constrained, in terms of mass seed production, by the prevention of the polar body release shortly after the fertilization. Prevention of the release of polar bodies is usually done through the provision of heat shock (Chourrout 1980), cold (Lemoine & Smith 1980), chemistry (Refstie et al 1977) and pressure (Piferrer et al 2009), with varying degrees of success ranging from 0 to 100% (Chourrout 1984). Not only the

success rate varies, but inconsistencies of the results were also found, even for the same method and sometimes a genetic mosaic was found (Nagler 2019). The shock treatment, especially heat shock, caused the zygotes' high mortality rate and decreased hatch rate by 97% (Prama et al 2018), with a success rate of tetraploidization of 77.66% (Prama et al 2019).

The latest breakthroughs can be pursued by tetraploid fish cross-breeding with diploid *P. hypophthalmus*, which is a collection from "Cabang Dinas Kelautan dan Perikanan Wilayah Utara (CDKPWU)", the West Java Fisheries and Marine Affairs. Thorgaard et al (1992) and Arai (2001) have been proven that triploid fish also can be produced by cross-breeding between tetraploid and diploid, with a success rate of 100%. The expected success of tetraploid and diploid fish cross-breeding activities can overcome the limitations of triploid fish seeds production, optimize growth uniformity, reduce feed conversion, reduce the duration of maintenance and meet the standard of filleting fish for export purposes. According to Zhou & Gui (2017), the polyploid genome of aquatic organisms has economic advantages due to a fast growth, broad adaptability and disease resistance. The purpose of this study was to prove that the cross-breeding of tetraploid with diploid *P. hypophthalmus* will produce 100% triploid *P. hypophthalmus* seeds. Then the next step was evaluating the cross-breeding production of triploid fish seeds, assessing the growth performance and growth of gonads, the yield of a fillet and a proximate of the triploid *P. hypophthalmus* meat.

Material and Method

Spawning and crossing of striped catfish broodstock. The tetraploid fish used is the result of previous research (Prama et al 2019). Four pairs were selected from each tetraploid and diploid *P. hypophthalmus* broodstock which have matured of gonads. The weight of each broodstock is 2.7-3.5 kg for the tetraploid and around 2.5-3.5 kg for the diploid broodstock. A total of 30 mg of tails were taken for identification of tetraploid broodstock by calculating the maximum number of nucleoli, based on the method of Howell & Black (1980) and Carman et al (1992). Before breeding, each broodstock is fasted for 24 hours and separately placed in different concrete ponds.

Ovulation induction, spermiation, egg incubation and larval rearing are following the method Prama et al (2018). Three cross-treatments were made, each consisting of 4 pairs, namely: (1) 2nF x 4nM (diploid female x male tetraploid), (2) 4nF x 2nM (female tetraploid x diploid male), and (3) 2nF x 2nM (diploid females and diploid males). Eggs incubation containers were used for observing the fertilization rate, hatching rate and larval survival. Three containers were used for ploidy analysis. Each observation used 200-300 eggs per treatment to calculate the degree of fertilization after 9 hours. The hatching rate was calculated by the volumetric method after 24 hours of egg incubation and the survival rate was calculated after two months of rearing.

Ploidy level analysis. A total of 100 larvae aged four days after hatching were taken from each cross-breeding for ploidy analysis. Ploidy analysis is done through observation of nucleoli of 500-600 cells per fish larvae (Carman et al 1992), chromosomes (Kligerman & Bloom 1977; Bencsik et al 2012) and confirmed by real-time quantitative PCR analysis. Genomic DNA extraction from 9 treated larvae was referred to Alimuddin et al (2007). PCR amplification was carried out using primers targeting growth hormone genes (GHF: 5 '- TGA T GG ATG ACT TTG AGG AAG - 3' and GF R: 5 '- CTC AAG GTC TGG TAG AAA TCC T - 3'), and β-actin (Cg βactin F 5'- ACC GGA GTC CAT CAC AAT ACC AGT-3 'and Cg β-actin R 5'- GAG CTG CGT GTT GCC CCT GAG-3') as internal standards (Nasrullah et al 2019). For the reaction of qPCR there were used machines Rotor-Gene 6000 (Corrbet, USA). Total final reaction volume of 20 µL, the qPCR program is set at 95°C for 2 minutes, followed by 40 cycles of 95°C for 5 seconds, 61°C for 20 seconds and 72°C for 20 seconds. The melting curve was analyzed after amplification to evaluate the primary specificity used. The difference in the ratio of the number of copies of GH-actin tetraploid and diploid fish was analyzed by the $2^{-\Delta\Delta CT}$ method (Livak & Scmittgen 2001).

Growth observation. Two months of old *P. hypophthalmus* seeds (total length: 1-2 cm) are identified as triploids using the nucleolus count method as described above. As a comparison, triploid and diploid *P. hypophthalmus* were kept in a 300 L fiber tub with a stocking density of 3 individuals L^{-1}, each one with three containers as replicates. The tubs were equipped with aeration systems to maintain dissolved oxygen content >3 mg L^{-1}. Feeding was administered ad-libitum for the first 10 days, using natural food such as bloodworms, then commercial feed (20% protein content) was given as much as 3% of the biomass weight, three times per day. Measurement of body weight and length wass done by taking a random sample of 30 individuals, once every 30 days. The growing period was of four months.

Gonad observations, fillet proportions, and meat quality. Observations on gonads, on the proportion of filet and on the meat quality were carried out on *P. hypophthalmus* aged two years. Male and female fish were taken both randomly (6 individuals of each). The fish was dissected, gonad was taken and weights measured, documented, then a few portions of the gonads were cut and put into 90% ethanol as much as 100 times for histological preparation (Fujimoto et al 2010). The skin is separated from the meat, then the filet is weighed. Furthermore, as much as 50 g filet was cut for proximate analysis based on SNI 01-2891-1992 (methodology of proximate analysis).

Statistical analysis. Data was tested using one-way ANOVA followed by Tukey's test to compare values between treatments, at $p<0.05$. Data analysis was performed with the help of Minitab 16 software.

Results

Reproduction performance. Reproductive performance of triploid *P. hypophthalmus* broodstock in the sense of fertilization rate (FR) and hatching rate (HR) are presented in Table 1. The values of FR and HR that use tetraploid broodstock are lower than cross-breeding diploid ($P<0.05$). Furthermore, the HR value between cross-breeding using the tetraploid broodstock was the same ($P>0.05$), but the HR value was lower in the cross-breeding 4nF X 2nM (7.01%) than 2nF X 4nM (26.77%).

Table 1

Fertilization rate (FR), and hatching rate (HR) of *Pangasianodon hypophthalmus* tetraploid cross-breeding X diploid and diploid X diploid

Breeding	*FR (%)*	*HR (%)*
2nF X 4nM	53.86 ± 12.23^{b}	26.77 ± 13.26^{b}
4nF X 2nM	50.66 ± 6.30^{b}	7.01 ± 1.08^{c}
2nF X 2nM	72.81 ± 3.33^{a}	51.22 ± 11.15^{a}

2nF-female diploid; 4nM-male tetraploid. Each crossbreeding consists of four pairs. Different superscript letters in the same column represent significant differences between treatments ($P<0.05$).

Ploidy determination. A total of 40 nucleoli have been found in all preparation samples, with a maximum number of three nucleoli in each cell; nucleoli ca be seen in Figure 1 (A); 81 chromosomes were counted, as in Figure 1 (B).

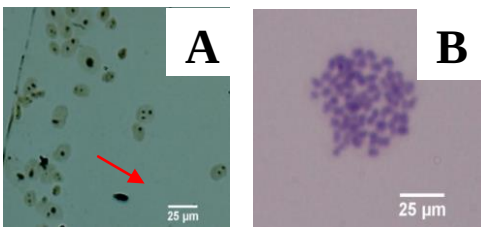

Figure 1. Nucleoli (A) and chromosome (3n=81) of *Pangasianodon hypophthalmus* triploid progeny.

The results of observations suggest a probability of 1.08±1.26% for the emergence of a maximum of three nucleoli from the progeny triploid striped catfish, with a total average of 519 nuclei. The percentage of maximum nucleoli emergence for each nucleus in the progeny triploid is shown in Table 2.

Table 2

The number of nucleoli and the ratio of the GH/actin gene in *Pangasianodon hypophthalmus* cross-breeding of tetraploid (4n) X diploid and inter diploid (2n)

Crossbreeding	*Percentage of nucleoli (%)*			*Ploidy level*
	1	*2*	*3*	
2nF X 4nM	74	25	1	all-triploid
4nF X 2nM	76	23	1	all-triploid
2nF X 2nM	79	21	-	all-diploid

2nF-female diploid; 4nJ-male tetraploid. Each crossbreeding consists of four pairs. Different superscript letters in the same column represent significant differences between treatments (P<0.05).

Observation of the ploidy level by counting the number of nucleoli and chromosomes shows that all larvae produced by cross-breeding are 100% triploid. Ploidy level confirmation using the qPCR method was performed on fish that were determined to weight around 30 g. One of 3 fish that had been confirmed triploid and diploid by the method of calculating the maximum number of nucleoli. As in Figure 2, the ratio of triploid GH/ actin gene was higher (p<0.05), compared to diploid.

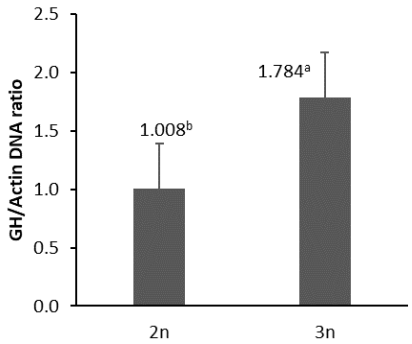

Figure 2. GH/actin DNA ratio in diploid (2n) and triploid (3n) *Pangasianodon hypophthalmus*, analyzed by quantitative real-time PCR method.

Figure 2 shows the concentration of the GH/actin ratio from the qPCR reading has an average value of 1.784 or 76.98% higher than diploid.

Growth performance. The rate of body length increase and feed conversion was better (p<0.05) on triploid fish compared to diploid, however, the rate of weight gain for triploid fish did not differ (P>0.05) from diploid fish (Table 3). The FCR values were 1.38 for triploids and 1.41 diploids, which are still relatively good even though they are maintained in a fiber container.

Table 3

Growth and survival of triploid progenies of *Pangasianodon hypophthalmus*

Parameter	*Triploid*	*Diploid*
SGR Length (% per day)	2.84±0.02[a]	2.61±0.14[b]
SGR weight (% per day)	8.12±0.04[a]	7.83±0.18[a]
FCR	1.38±0.00[a]	1.41±0.01[b]
SR (%)	100[a]	100[a]

Different superscript letters in the same line represent significant differences between treatments (P<0.05).

Gonad development. Observation with the histology of triploid *P. hypophthalmus* gonad at an age that was approved through gonadal maturity level. Male and female fish were used and the results of gonad histology were presented in Figure 3. Histology of male gonad triploid predominantly showed spermatocytes (Sc), and also spermatozoa (Figure 3A), whereas the diploid male gonad in Figure 3 (B) showed spermatozoa (Sz), followed by spermatocytes (Sc) and in a smaller proportion, by spermatogonia (Sg). Thus, it can be concluded that the development of a triploid male *P. hypophthalmus* gonad is stopped at the time of spermatocytes. This makes triploid male *P. hypophthalmus* proven not to produce gametes or infertile. Meanwhile, at that age diploid male *P. hypophthalmus* have produced functional gametes.

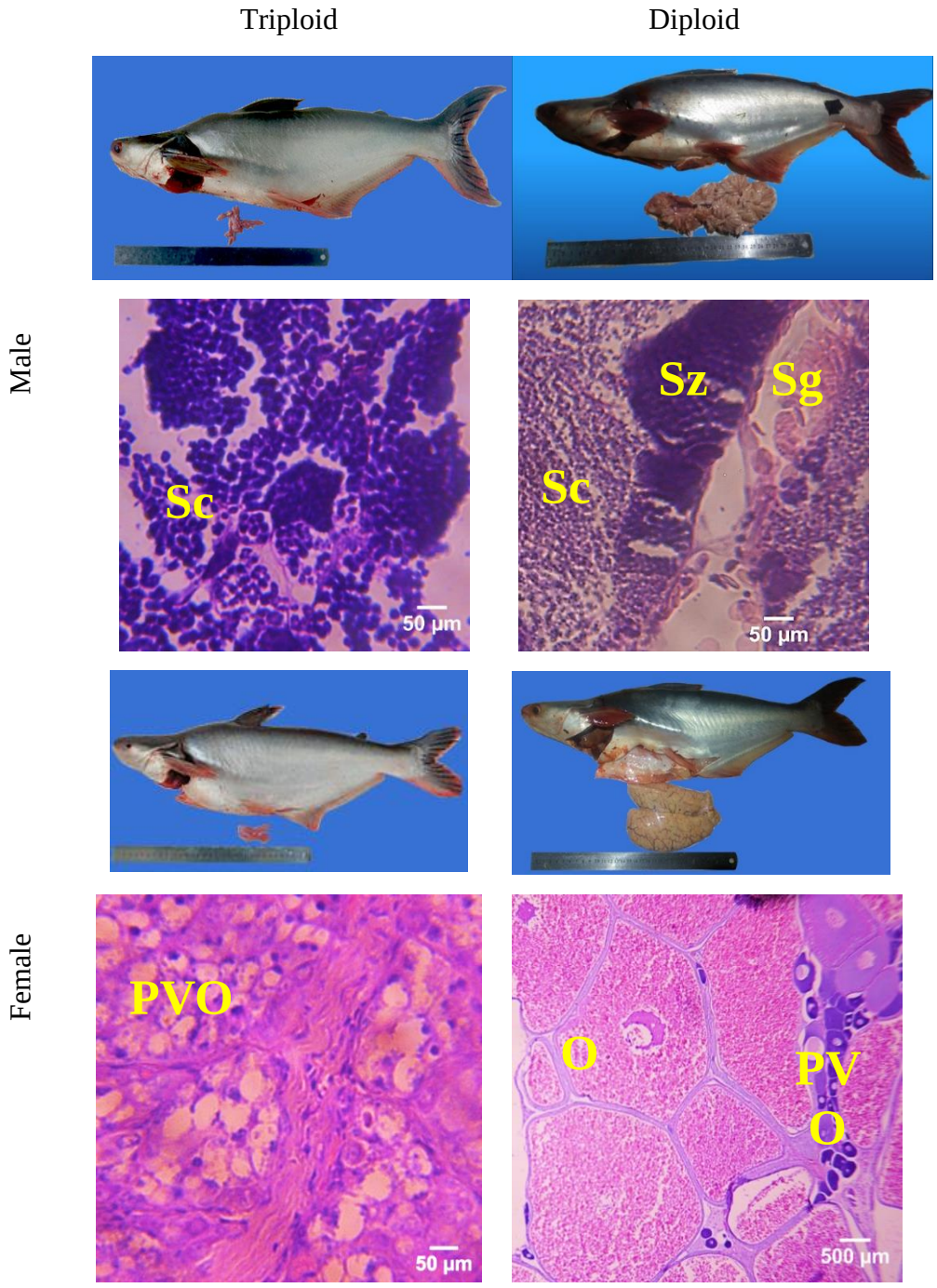

Figure 3. Histology of testis and ovaries of *Pangasianodon hypophthalmus* triploid and diploid at 33 months old.

Specifically for females, triploid *P. hypophthalmus* gonads stop and are dominated by eggs that have entered the previtellogenic stage. Also, female gonads do not develop to form functional gametes, iremaining infertile, while diploid female gonads are dominated by mature oocytes. However, there is a small percentage of eggs in the previtellogenic oocyte (PVO) and vitellogenic oocyte (VO) development stage. However, it is also necessary to further study the development of male and female gonads resulting from cross-breeding between tetraploid and diploid *P. hypophthalmus*, în order to determine whether they have the same gonadal developmental pattern when compared with induction triploids.

Fillet yield and quality. *P. hypophthalmus* are exported in the form of fillets. Therefore, the fillet yield is one of the important parameters to be measured. *P. hypophthalmus* used for filleting has an average weight of 2.24-3.48 kg. The results showed that the fillet yield of triploid females and males increased by 23.3% and 17.8%, respectively ($p < 0.05$), more than in diploid females and males (Table 4). This was certainly influenced by the gonad weights of developing diploid fish. Male and female *P. hypophthalmus* atfish both triploid and diploid are presented in Figure 3. Table 4 shows the result of the measurement of *P. hypophthalmus* fillet yield.

Table 4

Yield of *Pangasianodon hypophthalmus* triploid (2 n) and diploid (2n) male and female on 33 months

Sample	*Weight (kg)*		*Fillet weight (kg)*		*Fillet yield (%)*	
	Diploid	*Triploid*	*Diploid*	*Triploid*	*Diploid*	*Triploid*
Female	3.48±0.32	3.27±0.32	1.27±0.03	1.49±0.24	36.76 ±3.47^{b}	45.34 ±5.31^{a}
Male	2.31±0.62	2.24±0.31	0.88±0.27	0.99±0.12	37.79 ±1.74^{b}	44.50 ±0.96^{a}

Value is mean ± SD (n=6). Different superscript letters on the same line represent significant differences between treatments ($P<0.05$).

The fillet obtained as shown in Figure 4 (A) has pale yellow (light yellow) and 4 (B) reddish color.

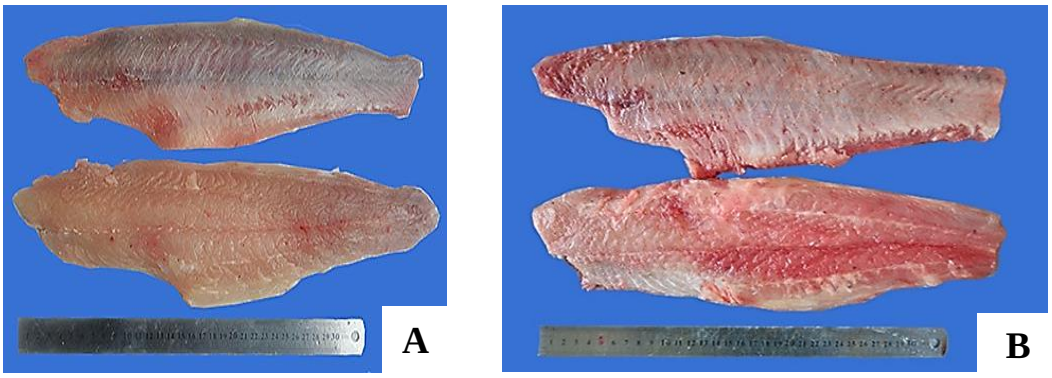

Figure 4. Fillet without fat and skin of triploid (A) and diploid (B) *Pangasianodon hypophthalmus*.

The difference in color on 4 (B) is due to a defective bleeding process, where the blood is still left in the flesh.

The quality of triploid meat. A portion of patin fillet meat has been taken to determine its proximate content. Proximate analysis results of *P. hypophthalmus* meat are presented in Table 5.

Table 5

Proximate content of triploid (3n) and diploid (2n) *Pangasianodon hypophthalmus* meat (% wet weight)

Sample	*Proximate composition (%)*					
	Moisture	*Ash*	*Protein*	*Fat*	*Rough fiber*	*NFE*
Triploid	74.92[a]	0.96[a]	20.19[a]	3.60[a]	0.10[a]	0.23[a]
Diploid	73.78[a]	1.00[a]	19.91[a]	4.71[a]	0.14[a]	0.46[b]

NFE-Nitrogen Free Extract. Different superscript letters in the same line represent significant differences between treatments ($P<0.05$).

The proximate content of triploid and diploid *P. hypophthalmus* meat is the same for all macronutrient components. Thus, the triploidization of *P. hypophthalmus* has a nutritional composition that is not different from that of diploid fish.

Discussion. Previous research on rainbow trout was reported that the second-generation triploid rainbow trout production by cross-breeding resulted in a FR of 40%, possibly due to larger sperm size which affected the fertilization rates (Chourrout et al 1986). Furthermore, the HR value was lower in the cross-breeding using tetraploid female broodstock (HR 7.01%). It indicates that egg quality affects more HR, although in this research there are not known factors yet that cause the quality of tetraploid broodstock eggs to be lower than diploid's. The quality of fish eggs can be defined as the ability of eggs to be fertilized, develop into normal embryos and hatch into larvae (Bobe & Labbé 2010; Migaud et al 2013).

The maximum percentage of emergence of pyrogenic triploid nucleolus is lower when compared with observations on the probability of nucleolus emergence made by Ibrahim (2016) of 2.49-13.15% for the maximum appearance of three nuclei in the induced triploid *P. hypophthalmus*. Besides nucleoli, observation and calculation of the chromosomes number from tetraploid fish are one method to ensure the success rate of polyploid induction activity (Pradeep et al 2011). To see the real differences between the two populations (diploid and polyploid), chromosome analysis is the most appropriate method (Nishiyama et al 2016). However, time spent on the sample preparation, observation and chromosome calculation is long and requires a special expertise (Faggio et al 2014).

Observation of number nucleoli to prove ploidy levels of fish is an appropriate method, with consistent results (Bencsik et al 2012; Ibrahim et al 2017; Daryanto et al 2019). This method is also easily carried out on fish ploidy upper-level larvae for a faster identification or by using the fish fins (Kim et al 2017). A large number of nucleoli is seen when staining using $AgNO_3$, due to the synthesis activity of ribosomes, producing proteins which are then blackened due to staining (Jankun et al 2007). According to Dunham (2004), nucleoli are formed during the process of transcription (synthesis of RNA) on cells. If the transcription process stops, the nucleoli disappear or shrink. So, nucleoli are not permanent organelles, which are needed as transcript marks to produce rRNA (ribosomal RNA) (Verdun 2011). Somatic cells, which rarely make synthesis proteins, will have small nuclei with solid chromatin and rarely have nucleoli, so nucleoli may be covered by chromatin masses (Jankun et al 2007) or merge into one large nucleolus (Carman et al 1992; Farley et al 2015).

The advantage of this GH/actin ratio measurement method can be done on fish aged a few days after hatching and does not torture the animal, because it uses fin samples. In addition to qPCR, ploidy analysis using flow cytometry is considered to have the advantage of being able to detect the total DNA genome on the cells with a large number of samples (Allen & Stanley 1983; Allen 1983). The flow cytometry method, although used as a verification tool, is commonly used to determine ploidy levels in polyploidization activities, but requires fish large enough to be able to take blood cells (Ewing et al 1991).

This method will certainly not be effective if the survival rate of polyploidized larvae results is low. Another weakness of the flow cytometry method is that sample

must be from uniform cells and may not be mixed with other cells, because it will interfere with reading results, so before it is measured, first the cell must be of a pure culture that requires time and cost (Ewing 1991). This shows that qPCR detect growth genes hormone which was previously widely used to see the zygosity/heterozygosity of transgenic fish (Alimuddin et al 2007; Marnis et al 2014, 2015, 2016; Kusrini et al 2016) can be used to distinguish ploidy levels on *P. hypophthalmus*. The β-actin genes in this analysis function as an internal control of genomic DNA content used as a template (Alimuddin et al 2007).

The same thing related to the survival and weight growth did not show significant differences during the initial grow up period, as it was also reported by Carter et al (1994); McGeachy et al (1995); O'Flynn et al (1997); Kalbassi & Johari (2008); Taylor et al (2011); Amoroso et al (2016) and Harvey et al (2017). However, after the second year of growing up, triploid turbot fish tend to start experiencing significant growth (Cal et al 2006) as does the rainbow trout (Janhunen et al 2018). FCR is very dependent on the media and quality of feed used during the rearing. Conversion rate of feed for *P. hypophthalmus* kept in swamp water ponds reaches 1.11-1.33, while for fish kept within floating net cage systems (KJA) immersed in rivers and lake waters it reaches 1.6-1.9 FCR. The most common FCR *P. hypophthalmus* cultivation in ponds can reach 1.59-1.70 (Solaiman & Sugihartono 2012). Survival rate data on similar studies did not show significant differences. Survival of triploid turbot fish after entering maturation stages consistently remained at 100%, while diploids began to experience death (Cal et al 2006). Triploid fish is a promising basic research model, despite the research reports regarding their weaknesses (Maxime 2008; Peruzzi et al 2018).

The histology of gonads taken from triploid *P. hypophthalmus* is influenced by the heat shock induction, in the study carried out by Ibrahim et al (2017). Approved evidence proving the gonad sterility of triploid *P. hypophthalmus* was obtained by observing the gonad development of the fish (Kalbassi et al 2013). Triploid *P. hypophthalmus* continue to produce eggs, but are blocked from entering maturation due to the disruption of the synapses due to an odd number of chromosomes (Pavlov et al 2013). Triploid mud loach fish have the same pattern of sterility as *P. hypophthalmus*, namely male and female gonads do not produce eggs and sperm is sterile and has a small size when compared with diploid gonads (Nam et al 2004). Likewise în the triploid turbot fish, but, despite being sterile, male gonads have almost the same size as diploids (Cal et al 2006). Unlike the case with Lincoln & Scott (1984) and Crisphalusi & Cloud (1999) reports, a study found that triploid male rainbow trout can produce sperm as diploid males, but the semen produced is runny (Benfey et al 1989) and most of it is affected by aneuploidy (Benfey et al 1989), but fish loaches couldsperm still can be obtained, which is able to move normally during activation (Zhao et al 2014). Specifically, some tetraploid females produce gonads that fail to develop into functional eggs due to failure to enter the meiotic stage (Nagler 2019). Lincoln & Scott (1984) also found that the size of the triploid rainbow trout male gonads had the same weight as diploid but the testes were dominated by spermatocytes. So, from the perspective of the gonad sterility of triploid fish, it is very suitable to develop these în order to restock ponds (Benfey 2015) and fishing ports (Lincoln & Scott 1984), which stands in particular for the pink salmon triploid, with special precautions to avoid escapes into the wild (Artamonova et al 2018).

An increase in the percentage of fillet in European seabass triploid fish were also reported by Peruzzi & Chatain (2003). Also, the female carcass was bigger than the males for all observed samples. Similar results were also found in yellow tetraploid fish, with a bigger carcass of females, compared to males (Nascimento et al 2017). According to Nam et al (2004), the appearance of triploid and diploid *P. hypophthalmus* fillet (skin and fat has been removed) is presented in Figure 4. The color in 4 (B) is determined by the bleeding process: here, the blood is still present in the flesh. The *P. hypophthalmus* fillet classification based on colors, released by The Vietnam Association of Seafood Exporters and Producers (VASEP), consists of four quality levels, namely: white (grade 1), pink (grade 2), light yellow (grade 3) and yellow (grade 4). Triploid fresh *P. hypophthalmus* without post-harvest treatment has entered grade 3, so it requires

treatment to bring the meat to a color grade 2 or 1. One effort to improve the color of *P. hypophthalmus* fillet meat is to cut off the blood vessels under the gill cover, so the blood is completely eliminated while the fish is still alive, then to wash the fillet meat with cold running water at 1°C (Akter et al 2014). The fillet meat color quality is also influenced by the level of gonad maturity: this is evident in triploid and diploid rainbow trout which are not mature and their immature gonads have the highest level of color quality, compared to specimens which have mature gonads (Janhunen et al 2018). This statement needs caution: in diploid *P. hypophthalmus*, the fillet has entered the level of perfect gonad maturation, while în the triploid *P. hypophthalmus*, the color quality of the fillet is not documented in detail.

The Norwegian Fisheries Industry Standard stipulates that *P. hypophthalmus* fish fillets can be divided into four types, depending on the preparation degree (scaling, trimming and gutting): Type A is a whole filet, without stomach, bones and skin; Type B is half of a filet, with fins and sometimes without ribs and half skinned (all or part of the silver stripe left on the fillet). Type C are weeded fillets, with bones, a thin abdomen and with the silver lining removed. Type D is a boneless fillet, without stomach and skin, where the silver lining and part of the brown muscle from the fillet have been removed. Fresh fillets of type B, C and D have usually been used. However, the type of fillet has been considered aș affecting the quality during the fillet storage, because some preparations still contain fatty tissue (untrimmed fillets) (Ikasari & Suryaningrum 2015).

Appearance, texture, taste, chemical content and food safety are parameters commonly used to determine the quality of fish fillets (Rario 2015). There are several grades used to distinguish the quality of fish fillets: the best quality is commonly referred to as grade A. Fish fillets classification as grade A depend on the species, especially for the taste and odor, and meet the minimum criteria set by U.S. Grade Determination, based on the physical and chemical structure of the fish flesh, color and degree of dehydration of the surface of the fillets, the cutting, scaling, trimming and gutting imperfections, the presence of bones and skin, and the texture of the fillet when cooked. However, all parameters that determine the quality of fish fillets are influenced by the primary processing of fish, including the process of catching or harvesting, cutting, the presence of blood, stomach contents, washing and filleting (Borderias & Alonso 2011). Of all these criteria, the most important thing is to properly handle and store, in order to avoid damaging or rottening (Akter et al 2014; Ikasari & Suryaningrum 2015).

Preparation for product filleting and storage should be performed: the fish in ice containers should be washed thoroughly with cold water (±1°C). Filleting is done in the post rigor stage to get a good and uniform fillet quality. The fillet is taken by cutting the meat from one side of the spine lengthwise from the tail to just behind the head, then doing the same thing on the other side. Filleting has been done manually using a sharp and thin knife. Immediately after filleting, wash with cold water (±1°C) to remove blood, the contents of the stomach and especially the kidneys and bile, which can affect the texture and appearance of the product. The fillet is then packaged using a polyethylene bag and stored in a freezer at -20°C. Handling hygienic techniques of fillets should refer to the standard guidelines in each export destination country (Akter et al 2014). Filleting can also be operated on fresh fish that are still alive by cutting off their headand by hanging them to drip their blood for 10 minutes. The filleting process can be performed aș in the procedure described above (Ikasari & Suryaningrum 2014).

The results of previous studies related to proximate composition and color of meat having the same values between triploids and diploids have been reported in shi drum fish (*Umbrina cirrosa* L.) (Segato et al 2007). Conversely, it has been reported that triploid fish fat content is lower than în the diploid fish, except for the spawning season. In the reproductive season, the fat levels are the same (Peruzzi et al 2018; Haffray et al 2005; Werner et al 2008). Murray et al (2018) presented data on total fat in Atlantic triploid salmon fillets, which are significantly lower, compared to diploid fish. Regarding the turbot meat quality and texture, Ayala et al (2020) stated that their values în diploid fish specimens are higher than in triploids.

Conclusions. The current research concludes that tetraploid *P. hypophthalmus* can be crossed-with diploid *P. hypophthalmus* for the mass production of triploids, without physical shock treatment on zygotes. Crossbreeding of male tetraploid *P. hypophthalmus* with diploid female determine better fertilization levels and hatching rates than the crossbreeding of tetraploid females with diploid males. Regarding the growth performance, the weight growth and survival of seeds did not show significant results on, but the length growth and FCR value had a significant progression. Histology results showed that triploid gonads were permanently infertile, while diploids produced sperm and eggs that could reach maturity and thus they were sexually ready for spawning. Triploid striped catfish fillet is 6.70-8.58% larger and has better nutritional value than diploid, interms of protein, fat and nitrogen free extract.

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