

## Expression of the Growth Hormone Gene during Early Development of Tiger Shovelnose Catfish (*Pseudoplatystoma Fasciatum* Linnaeus, 1766)

Erma P. Hayuningtyas<sup>1,2,4</sup>, Abinawanto<sup>2</sup>, Eni Kusri<sup>1,4</sup>, Agus Priyadi<sup>1,3</sup>, Ratu Siti Aliah<sup>4</sup>

<sup>1</sup> Research Institute for Ornamental Fish Culture, Ministry of Marine Affairs and Fisheries, Depok, West Java, Indonesia

<sup>2</sup> Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok, West Java, Indonesia

<sup>3</sup> Research Center for Conservation of Marine and Inland Water Resources, National Research and Innovation Agency (BRIN), Jakarta, Indonesia

<sup>4</sup> Research Center for Fishery, National Research and Innovation Agency (BRIN), Jakarta, Indonesia

**Abstract:** The early development stage of fish is crucial in fish farming, especially catfish species with cannibal characteristics, such as the Tiger Shovelnose Catfish (*Pseudoplatystoma fasciatum* Linnaeus 1766). Growth plays an essential role in fish's life, which is influenced by the mechanism of growth hormone (GH). This study analyzed the level of growth hormone (GH) gene expression at several early developmental stages of Tiger Shovelnose Catfish. Samples were taken from an embryo, three days post hatched (dph) larvae to 60 dph juvenile. Isolation of mRNA was carried from the pituitary gland located at the head of the fish. Gene expression analysis was performed using RT-PCR method that compared GH/ $\beta$ -actin genes from band thickness. The results of the analysis showed the highest expression at 60 dph juvenile. The most significant increase in GH gene expression occurred from the egg to three dph larvae ( $P < 0.05$ ), then decreased to 20 dph juvenile. The increased phenotypic growth was seen from the increased length and weight at 45 dph juveniles. This indicates the amount of growth hormone released at the juvenile stage of 20 dph, thus triggering rapid growth in the next stage at 45 dph juvenile. Growth hormones can act as growth biomarkers in Tiger Shovelnose Catfish. The novelty of this study was to determine the level of GH gene expression in *Pseudoplatystoma fasciatum* ornamental fish as the object of research. Suggestions for further research to explore genes related to growth mechanisms through transcriptomic and proteomic technologies.

**Keywords:** mRNA, biomarker, molecular biology, aquaculture, growth performance.

## 虎鲶鱼(假性筋膜口囊肿林奈, 1766) 早期发育过程中生长激素基因的表达

**摘要:** 鱼类的早期发育阶段对鱼类养殖至关重要,尤其是具有食人特征的鲶鱼,如虎鲶鱼(假性筋膜口囊肿林奈176)。生长在鱼的生命中起着至关重要的作用,它受生长激素机制的影响。本研究分析了虎铲鼻鲶几个早期发育阶段的生长激素基因表达水平。样品取自胚胎,孵化后3天至孵化幼虫后60天。从位于鱼头部的垂体中分离出信使核糖核酸。使用逆转录聚合链反应方法进行基因表达分析,该方法从条带厚度比较生长激素/ $\beta$ -肌动蛋白。分析结果显示在孵化后60天的幼鱼表达量最高。生长激素基因表达的最显著增加发生在卵到孵化后3天("NUM0"),然后下降到孵化后20天的幼体。从孵化后45天幼鱼的长度和体重增加可以看出表型生长的增加。这表明在孵化后20天的幼体阶段释放的生长激素量,从而

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About the authors: Erma P. Hayuningtyas, Research Institute for Ornamental Fish Culture, Ministry of Marine Affairs and Fisheries, Depok, Indonesia; Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok, Indonesia; Research Center for Fishery, National Research and Innovation Agency (BRIN), Jakarta, Indonesia; Abinawanto, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok, Indonesia; Eni Kusri, Research Institute for Ornamental Fish Culture, Ministry of Marine Affairs and Fisheries, Depok, Indonesia; Research Center for Fishery, National Research and Innovation Agency (BRIN), Jakarta, Indonesia; Agus Priyadi, Research Institute for Ornamental Fish Culture, Ministry of Marine Affairs and Fisheries, Depok, Indonesia; Research Center for Conservation of Marine and Inland Water Resources, National Research and Innovation Agency (BRIN), Jakarta, Indonesia; Ratu Siti Aliah, Research Center for Fishery, National Research and Innovation Agency (BRIN), Jakarta, Indonesia

在孵化后45天的幼体引发下一阶段的快速生长。生长激素可以作为虎鲶的生长生物标志物。本研究的新颖之处在于确定作为研究对象的假性筋膜口囊肿观赏鱼的生长激素基因表达水平。建议进一步研究通过转录组学和蛋白质组学技术探索与生长机制相关的基因。

**关键词：**信使核糖核酸, 生物标志物, 分子生物学, 水产养殖, 生长性能。

## 1. Introduction

Tiger Shovelnose Catfish (*Pseudoplatystoma fasciatum* Linnaeus, 1766) is an introduced ornamental fish originating from the Amazon River [1]. This fish from the genus *Pseudoplatystoma* has eight species identified in its home country of Brazil. Species of the genus *Pseudoplatystoma* that have been successfully cultured in Indonesia are *Pseudoplatystoma fasciatum* [1, 2]. This fish is an exported ornamental fish commodity and much-loved by hobbyists because of its predatory nature and large size. The growth of this fish is also relatively fast, especially in the early development, which tends to behave in cannibalism [1]. Research on rapid growth at the beginning of fish development genetically has not been widely carried in cannibal fish, particularly in the *Pseudoplatystoma* genus. The determining factor in the growth of this fish is Growth Hormone (GH). Therefore, growth hormone is important in the sustainability of fish growth.

Growth hormone (GH) is a biomarker used to characterize growth. The anterior pituitary gland secretes this hormone in each individual [3, 4]. The growth hormone is naturally only expressed in the pituitary gland [5]. Growth hormone plays an essential role in stimulating fish growth of its cognate receptor, protein biosynthesis, key nutrient transporters in the intestine and feed conversion [6–8]. Additionally, growth hormones can modulate or act as adaptive hormones to environmental conditions, such as pollutant, temperature, salinity, stress, and apoptosis [4, 9–11]. Growth hormone can also change fish behavior because it can increase appetite, aggressiveness, swimming activity, and anti-predator. Additionally, this hormone affects metabolism, reproduction, feed conversion, and body immunity [7, 8, 11]. Fish growth is influenced by environmental factors such as temperature, photoperiod and food availability. It is also genetically controlled by hormones. The production of growth hormones that affect photoperiod also affects fish maturation, such as gonadal development, gametogenesis, and reproductive cycles [9].

Growth hormone has two types of mechanisms, namely, direct and indirect. The indirect agent is due to the mediation of insulin-like growth factor (IGF-1) secretion in the liver [12, 13]. Growth hormone is directly secreted by the pituitary gland, bound by binding proteins, and then expressed to several target

tissues [3]. Research related to GH gene expression has been widely carried out in several fish commodities such as tilapia [5], Mozambiquen tilapia [4], African catfish [14], goldfish [7] and globally specifically investigated GH gene expression in the early developmental stages of [10], cobia fish [12], and Siberian sturgeon [3].

The expression of the GH gene in the early stages of fish development was investigated to determine the genetic mechanism of growth hormone in fish. Research on gene expression in fish of the *Pseudoplatystoma* genus has also been carried out at the early stages of fish development. In the *Pseudoplatystoma punctifer* species, gene expression related to digestive enzyme activity can reveal omnivorous behavior even though these fish are carnivorous fish [15]. Previous studies on GH gene expression in the early stages of Siberian sturgeon fish have been conducted using RT-PCR and real-time PCR methods [3, 16]. This fish has a large size and fast growth like the Tiger Shovelnose Catfish. Growth in the early stages is a key to culture Tiger Shovelnose Catfish. However, information related to GH gene expression in the early development of Tiger Shovelnose Catfish is still minimal, so it becomes an exciting thing to study in the field of molecular biology. This study analyzed the expression level of growth hormone (GH) genes at several early stages of development of Tiger Shovelnose Catfish (*Pseudoplatystoma fasciatum* Linnaeus, 1766).

## 2. Material and Method

### 2.1. Sample Preparation

Sample collection for GH gene expression was carried out at several early developmental stages of Tiger Shovelnose Catfish (Fig. 1). The research material in Tiger Shovelnose Catfish was obtained from ornamental fish farmers at the “Gumelar’s Farm,” a Small-Scale Hatchery. Tiger Shovelnose Catfish have been spawned artificially or induced breeding with the gonadotropin hormone stimulation [1]. Spawning was carried out with a male and female ratio of 1:1, producing fertilized eggs. The female parent weighed 3.6 kg, and a total length of 71 cm was used, while the male parent weighed 4.9 kg and a full length of 79 cm. Each was injected with the gonadotropin hormone

Ovaprim® (Syndel, Canada) at a dose of 0.6 mL/kg and 0.8 mL/kg.

After fertilization, the eggs were incubated in an aquarium measuring 85 × 40 × 20 cm filled with ± 10 L of water. About 1000 eggs were stocked in each aquarium for incubation. Sampling was carried out at the embryonic stage, 3 dph, 10 dph, 15 dph larvae stage, and 20 dph, 45 dph, 60 dph juvenile stage. The sampling schedule was determined based on the early development stage and feeding frequency by fish farmers (Fig. 2).

Growth measurements were carried out at each stage of early development in the form of weight and full-length sampling. At the embryonic stage, the measurements of the weight and diameter of the eggs were conducted. Before measures, the fish were anesthetized using Phenoxyethanol at a 200 µL/L of water dose. After the fish were unconscious, measurements were then taken. Another observed parameter was the pituitary tissue for the growth hormone gene expression.

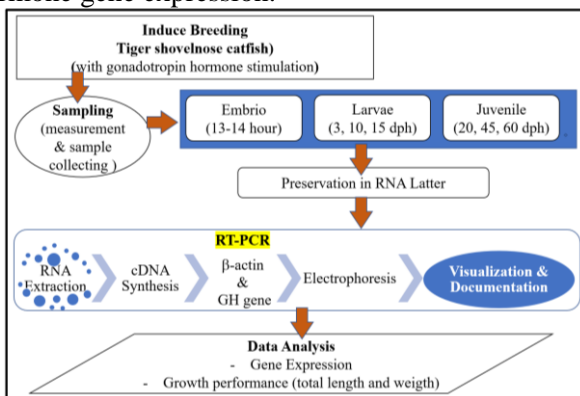


Fig. 1 Flowchart of research methodology in Tiger Shovelnose Catfish (*Pseudoplatystoma fasciatum*)

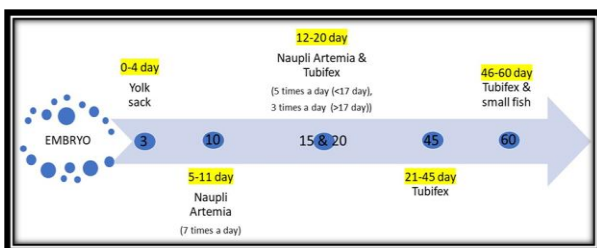


Fig. 2 Feeding scheme in the early development of Tiger Shovelnose Catfish (*Pseudoplatystoma fasciatum*) in the "Gumelar's Farm" a Smale-Scale hatchery

Note: The numbers on the arrows indicate the sampling timeline; information outside the arrow line is the range of maintenance time and the type of feed given at the stage.

## 2.2. RNA Extraction and cDNA Synthesis

Sampling was carried out in three repetitions with the provision that the sampling for the embryo stage was 30 eggs per sample with an average diameter of 1.3 mm, and three dph larvae, i.e., three individual per sample, while for 10 dph larvae to 60 dph juvenile, i.e., one individual per sample. Samples were preserved using RNA Litter (Qiagen). In the embryo, the number of pull samples used 30 eggs because, during the

preliminary study, the total RNA concentration was very low, using 5–10 eggs. Therefore, either  $\beta$ -actin or GH genes could not be detected when PCR was carried out. The larvae samples were first anesthetized using Phenoxyethanol at a 200 µL/L water dose. Only the head was taken in a sample weighing more than 50 mg, or the brain and pituitary organs were taken. Then, tissue samples were weighed, and RNA was extracted using 1 ml Tri reagent Kit (MRC) according to the manufacturer's protocol. After obtaining the total RNA, the cDNA synthesis process was continued using the NZY First-Strand cDNA Synthesis Kit (Nzytech). The premix composition for cDNA synthesis: NZYRT 2× Master mix 10 µL, NZYRT Enzyme Mix 2 µL, Total RNA concentration of 1 ng, and DEPC – treated H<sub>2</sub>O up to a total volume of 20 µL. The premix solution was mixed, incubated at 25°C for 10 minutes, 50°C for 30 minutes, and inactivated the reaction at 85°C for 5 minutes. The NZY RNase *E. coli* was added 1 µL and incubated at 37°C for 20 minutes. The cDNA samples were stored at -20°C.

## 2.3. GH Gene Expression by RT-PCR Method

Expression of growth hormone (GH) genes in Tiger Shovelnose Catfish was carried out by PCR for housekeeping genes in the form  $\beta$ -actin (Kusrini et al., 2018) and degenerate primer catfish GH target genes [17].

### 2.3.1. Detection of $\beta$ -Actin Gene

The  $\beta$ -actin gene was detected as a housekeeping gene using the RT-PCR method with the Dream Taq Master mix Green Kit (ThermoScientific). Reaction compositions based on the kit protocol: 12.5 µL master mix, 1 µL primer  $\beta$ -actin F, 1 µL primer  $\beta$ -actin R (concentration of 20 pmol each) (Table 1.), 1 µL cDNA (1000 ng/µL), and *nuclease-free water* up to a total volume of 25 µL. PCR amplification conditions with the program were initial denaturation temperature of 94°C for 3 minutes, denaturation temperature of 94°C for 30 seconds, annealing temperature of 58°C for 30 seconds, and extension temperature of 72°C for 30 seconds. This cycle at was repeated 35 times, terminated with a final extension of 72°C for 3 min, and final conditioning at 4°C.

Table 1 Primer sequence, length, GC content, temperature melting (TM), and size target of primer

No.	Primer	Sequence (5'-3')	Length	GC (%)	TM (°C)	Size (bp)
1	GHC F1	CAG GCA AGG	21	61,9	61	234
		ACG AGA CCC				
	GHC R1	AGA	24	58,33		
		CTG GTA GAA				
2	$\beta$ -actin F	ATC CTC GAA	20	75	58	300
		GGG CGG				
	$\beta$ -actin R	GGT CGA GCT	20		58,33	
		GGA CGG CGA				
		CG				
		ACG AAC TCC				
		AGC AGG ACC				
		AT				

The GH gene was detected using the RT-PCR method with the Dream Taq Master mix Green (ThermoScientific) Kit. The results from the optimization of the degenerate primer GH catfish in a previous study were used in the primer. The degenerate primer was designed using seven catfish sequence data in the Gene Bank (NCBI) from 5 fish species, namely *Heteropneustes fossilis* (AF147792.1), *Pangasianodon gigas* (L27835.1), *Pelteobagrus fulvidraco* (DQ112163.1), *Ictalurus punctatus* (NM\_001200245.1), and three sequence data from *Clarias gariepinus* (FJ823972.1, MN249238.1, EF411172.1). Reaction composition based on Kit Dream Taq Mastermix Green protocol (ThermoScientific) were 12,5 µL master mix, 1 uL primer GH F1, 1 uL primer GH R1 (concentration of 20 µmol each) (Table 1), 1 µL cDNA (1000 ng/µL), and *nuclease-free water* up to a total volume 25 µL. PCR amplification conditions with the program were initial denaturation temperature of 94°C for 3 minutes, denaturation temperature of 94°C for 20 seconds, the annealing temperature of 62°C for 30 seconds, and extension temperature of 72°C for 30 seconds. This cycle was repeated 40 times, terminated with a final extension of 72°C for 7 min, and final conditioning at 4°C.

2.3.2. Electrophoresis and Visualization

The results of amplification of the β-actin and the GH gene were electrophoresis on 1.5% agarose gel with 1x TBE, which was immersed in 0.5 × TBE solution. A volume of 5 µL of PCR product was inserted into the electrophoresis well, parallel to 2 µl of DNA ladder 100 bp plus (Vivantis) as a marker. Electrophoresis was performed at 100-volt voltage for 45 minutes. The electrophoresis results were visualized

and documented on a Gel Doc UV transilluminator (Simple Protein).

2.4. Data Analysis

Gene expression analysis was conducted in semi-qualitative. The analysis was carried out by comparing the expression ratio between GH and β-actin genes from each stadia observed. The quantification of the resulting fragment thickness was analyzed using Alpha View SA software. The data GH/β-actin gene expression ratio was presented descriptively in the form of a bar chart. Statistical analysis was performed to compare the GH gene expression at several developmental stages using one-way ANOVA and Tukey’s post hoc HSD analysis. Data analysis was performed using the SPSS software. Growth performance data based on body weight and total length were analyzed descriptively and presented in a graphical form.

3. Result

Total RNA isolation results from Tiger Shovelnose catfish samples showed concentrations ranging from 221 – 2862 ng/µL according to the number and samples used. RNA purity was measured at an absorbance of 260/280 with a purity range of 1.8 – 2.0. The highest concentration of total RNA was produced in the embryo sample, while the lowest one was in the ten dph larvae (Table 2).

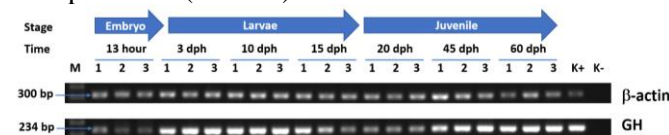


Fig. 3 PCR product amplification of β-actin and GH gene from embryo to juvenile stage

Table 2 Total RNA isolation data of tissue types, amount, concentration, and purity from each stage

Stage	Organ tissue	Number of samples	Concentration (ng/µL)	Purity (A260/280)
Embryo	Egg	30 eggs	2567 ± 311.6	1.841 ± 132.1
Larvae 3 dph	Whole larvae	5 larvae	401 ± 150.2	1.817 ± 13,4
Larvae 10 dph	Whole larvae	1 larvae	299 ± 72.7	1.848 ± 13,6
Larvae 15 dph	Whole larvae	1 larvae	623 ± 114.3	1.838 ± 5.1
Juvenile 20 dph	Head	1 juvenile	697 ± 219.4	1.872 ± 50
Juvenile 45 dph	Brain and pituitary	1 juvenile	1101 ± 284.1	1.880 ± 105
Juvenil 60 dph	Brain and pituitary	1 juvenile	861 ± 230.7	1.849 ± 42.8

The electrophoresis of the amplification of the β-actin gene and the GH gene is shown in Fig 3. The appearance of the β-actin gene band was visible at the size of 300 bp. The GH gene appeared at 234 bp in length. The thickness of the band varied from individual to individual. The thickness of the β-actin gene band appeared more consistent than the GH gene band because this gene acted as an internal control of GH gene expression. The thinnest visible band was in the positive control, which was a sample of the pituitary tissue of adult Tiger Shovelnose Catfish. The

thickness of the bands in the GH gene had a different pattern and looked more varied because there were thin bands, and some were very thick bands. The embryonic stage of the GH gene produced had a narrow band, then increased in the larval stage the thickness of the band became thicker. However, at the end of the larval stage to the beginning of the juvenile stage, there was a depletion of the expressed GH band and it increased again at the stage of 45 dph juvenile. The results of the calculation of gene expression levels or the ratio of the GH gene/β-actin gene are shown in Fig. 4.

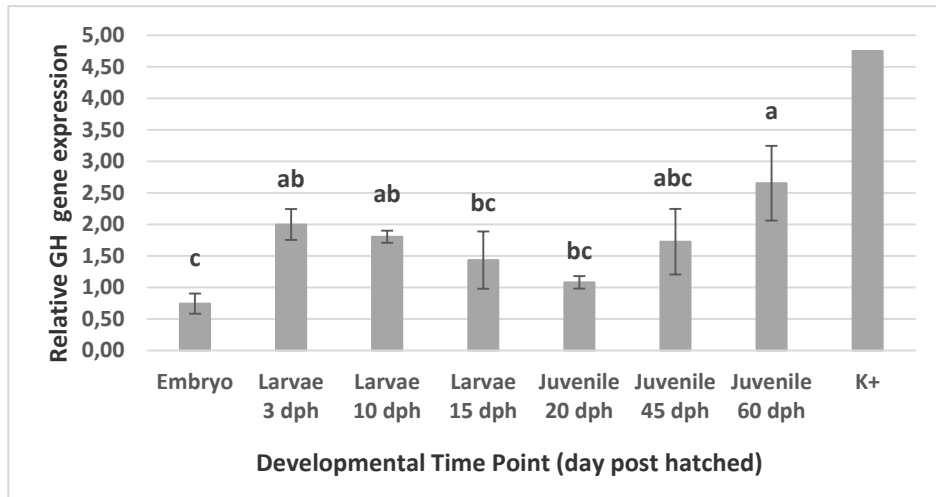
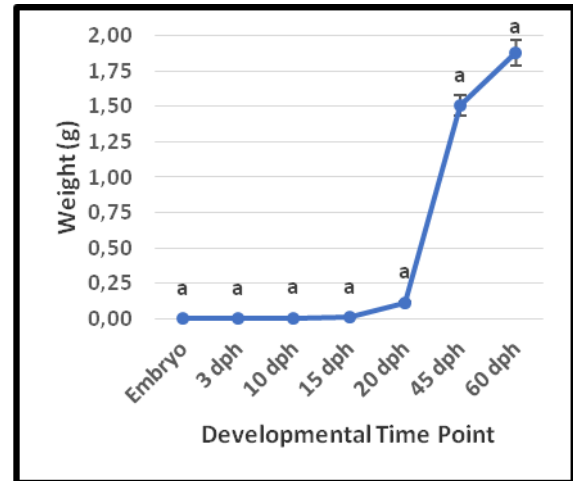


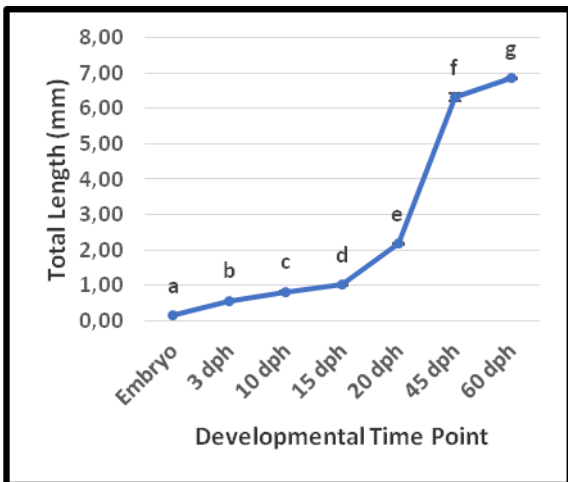
Fig. 4 Relative GH gene expression levels (GH/β-actin ratio) of GH during early development of Tiger Shovelnose Catfish with RT-PCR method

The level of GH gene expression for all samples observed from each stage was detected. GH gene expression at the embryonic stage had the lowest level compared to other stages. An increase in the expression level at the 3 dph larvae stage happened, but the expression level decreased again until the stage was 20 dph juvenile. Then, increased levels of GH gene expression began to occur at the stage of 45 dph to 60 dph juvenile. The GH gene expression level at 60 dph juveniles was the highest peak of all observed expression levels, but its expression was still much lower than the GH gene expression in adult fish (K+).

The results of the observed growth performance of Tiger Shovelnose Catfish are shown in Fig. 5A, based on total length and 5B based on body weight. Fish growth at the larval stage did not show a significant increase based on body weight. Based on the total length, the increase in growth continued to occur significantly ( $P < 0.05$ ). Growth increases most markedly after entering the juvenile stage. A significant increase in growth was registered at the 45 dph juvenile stage, both from the total length and weight of the Tiger Shovelnose Catfish.



(b)  
Fig. 5 Growth performance of the early development in Tiger Shovelnose Catfish (*Pseudoplatystoma fasciatum* Linnaeus, 1766), average total length (cm) (A), and average weight (g) (B). Different letters indicate significant different values at  $P < 0.05$



(a)

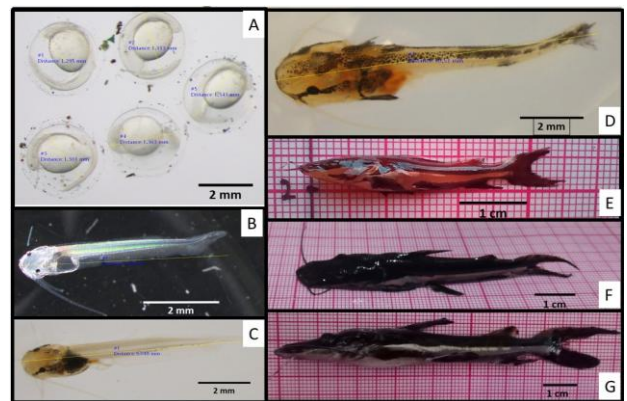


Fig. 6 Phenotypes of some early developmental stages of Tiger Shovelnose Catfish. Note: a) embryo; b) 3 dph larvae; c) 10 dph larvae; d) 15 dph larvae; e) 20 dph juvenile; f) 45 dph juvenile; 60 dph juvenile

Metamorphosis or phenotypic changes in shape from each stage of the Tiger Shovelnose Catfish can be seen in Fig. 6, Fig. 6A shows the embryo in the final

phase before hatching, commonly called the eye phase. However, the pigmentation in the eye of the fish was not observed because the larvae in the embryo were still transparent and had no visible pigmentation. Pigmentation in 3 dph larvae was visible in the eyes, but the body surface was still acceptable (Fig. 6b). Pigmented ten dph larvae began to form around the head (Fig. 6c). In 15 dph larvae, pigmentation was seen around the back of the body to the tail (Fig. 6d). The Fig. 6E image entered the stage 20 dph juvenile, the fish phenotype was seen to have more complete organs, and pigmentation was almost formed in all body parts. At 45 dph, the juvenile had a darker skin tone and was starting to create a pattern of spots and stripes (Fig. 6F). However, 60 dph juvenile showed that the larger the size, the more complete the organs of the body and the more formed the pattern was (Fig. 6g).

#### 4. Discussion

The GH gene detected for several stages showed different gene expression at each stage. The difference in expression depends on the stadia, age, and the type of sample used (Fig. 3). The individual samples represent all stages of biological development, starting from embryos, larvae, and juveniles at various ages. The results of this study follow the results of research on several fish species Siberian sturgeon (*Acipenser baerii*) [3], and Japanese eel (*Anguilla japonica*) [17], Mozambiquen tilapia (*Oreochromis mossambicus*) [10], which began to be expressed in the embryonic stage. The expression of the GH gene in Tiger Shovelnose Catfish embryos is thought to be due to the formation of the pituitary gland in these embryos. According to Abdolahnejad et al. [3], GH expressed in Siberian sturgeon fish embryos is thought to be due to the formation of the pituitary gland before the embryo hatches and the presence of endocrine activity in these fish. Thus, it greatly affects the function of growth hormones in the fish. The growth hormone in fish plays a role as the main promoter in growth that affects metabolism and changes fish behavior, such as increasing appetite, swimming activity, aggressiveness, and reducing anti-predatory behavior [18].

Increased expression levels in Tiger Shovelnose Catfish began to occur on the third-day post-hatching (dph) larvae with a higher expression level than at the embryonic stage. This was different from what happened in the Siberian sturgeon GH gene expression decreased compared with the embryonic stage until 20 dph juvenile, then increased expression at 25 dph. These results were also different from those of milkfish, where the increase in gene expression continued to occur with the increasing age of the fish but began to decrease on the tenth day after hatching. This pattern is similar to that of the Tiger Shovelnose Catfish in that there is a decrease in the GH gene expression at 10 dph larvae. The expression level

continued to decrease to 20 dph and again increased to 45 dph juvenile. The increase still occurred up to 60 dph; it is suspected that the increase in the GH gene expression will continue to increase until adulthood. According to Abdolahnejad et al. [3], and Zhong et al. [5], growth hormone genes can only be expressed naturally in the pituitary gland from the early stages of life development until adult fish. Zhong et al. [5] discovered that in normal adult fish, the detection of GH genes is carried out in organs such as muscles and pituitary liver, spleen, intestines, brain, ovaries, testes, kidneys, and liver, but the gene is only detected and expressed in the pituitary organ. However, the GH gene can also be expressed in other organs, such as in transgenic fish. The GH gene is expressed throughout the body of transgenic fish; this was proven by the success in isolating the GH gene from the fin tissue of several transgenic fish such as Common carp [19], Betta fish [20], Coho salmon [21], and African catfish [14]. Additionally, the GH gene is also expressed in sperm produced by transgenic fish [14, 19].

The growth performance of Tiger Shovelnose Catfish-based on body weight did not increase significantly ( $P>0.05$ ), but based on full-length, it always experienced a significant increase ( $P<0.05$ ). Growth hormones play heterologous and homologous roles in increasing specific growth based on length and weight in Teleostei [18]. The growth in length and weight in Tiger Shovelnose Catfish began to increase after entering the juvenile stage, a metamorphosis stage for fish at 20 dph juvenile. At the 20 dph juvenile stage, gene expression was low compared to the larval and juvenile stages. It is suspected that the GH gene plays an important role in metamorphosis from larvae to juvenile so that its expression decreases at 20 dph juvenile stage. The same thing also happened to Siberian sturgeon in its metamorphosis phase, which was reduced GH gene expression on days 15–20 dph [3]. Phenotypically, changes in body shape also occurred at this stage with pigmentation that began to spread evenly throughout the body, and the morphology started to form from larvae to small fish or juveniles. Here, the decrease in the GH gene expression is thought to occur directly, through the mechanism of releasing GH protein by the pituitary and circulating to target organs with the help of growth hormone-binding proteins [13].

The increase in growth of Tiger Shovelnose Catfish occurred significantly at 45 dph accompanied by an increase in GH gene expression. This indicates that the amount of growth hormone released at the juvenile stage of 20 dph triggered rapid growth in the next stage at 45 dph. Phenotypically, the pigmentation and shape of the body and organs were more perfect than before. Fish growth is polygenic, controlled by the environment and influenced by growth hormone (GH), and insulin-like growth factor-1 genes. These genes

function to regulate the Hypothalamic Pituitary Somatotrophic (HPS) endocrine pathway [13]. The endocrine pathway in fish is almost similar to that in humans, the IGF-1 gene is produced in the liver and GH as a stimulus to synthesize into the blood circulation [11].

The increase in weight and length is thought to be due to the induction of the GH gene, which functions in increasing fish appetite and affects the feed conversion [14]. Evidence was the increase in the growth of some transgenic fish induced by the GH gene resulting in several times higher weight growth than normal fish [14, 16, 19]. For example, in the case of transgenic catfish, feed conversion can affect feed efficiency, protein content, and higher essential amino acid content than normal fish [14]. However, both normal and transgenic fish need proper and balanced nutrition to support their growth. Supplementation in the form of recombinant growth hormones in feed can also stimulate growth in fish and is not genetically inherited as in transgenic fish. The addition of a recombinant growth hormone to commercial feed can accelerate the growth performance in normal fish [8].

The highest expression level of growth hormone gene in the early stages of development of Tiger Shovelnose Catfish was at 60 dph juvenile. At this stage, both the size of the weight and length and the morphology were complete, resembling an adult fish in a smaller size. This indicates that the metamorphosis process has ended so that the energy used was focused on the growth of weight and length, instead of on the formation of organs, pigmentation, etc. Thus, it is suspected that the presence of GH gene expression is higher because it is not shared with other mechanism functions in the fish development cycle. Increased expression of GH genes at the juvenile stage has also been reported in Siberian sturgeon fish with high expression levels at the stage of 25 dph to 50 dph juvenile [3]. With increasing age, the expression of GH genes is higher at the juvenile stage, with organs that are phenotypically more complete.

## 5. Conclusion

This study concluded that the expression of growth hormone genes in the early stages of development of Tiger Shovelnose Catfish can be detected from the embryonic stage. GH gene expression levels decreased from larva to juvenile at the metamorphosis stage, the highest expression at the late juvenile stage. We used a fast growing Tiger Shovelnose Catfish (*Pseudoplatystoma fasciatum*) as the object of study, knowing the level of GH gene expression in the early stages of growth is a novelty of this research and the perspective research in the future. The limitation of this study is that it uses the semi-quantitative RT-PCR method to determine the GH gene expression. Suggestions for further research are to use quantitative methods such as qPCR for gene expression analysis

and explore gene mechanisms through transcriptomic and proteomic technologies.

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