The effect of gradual decrease in pH level on the survival rate and glucose levels of catfish (*Pangasius* sp.)

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Abstract. *Pangasius* sp. is a superior hybrid catfish with the characteristics of premium quality meat, which has good adaptability and availability (continuity and quantity) of seeds throughout the season. In Southeast Asia, especially in Indonesia, it is possible to develop catfish farming on marginal peatlands. The current study was designed to determine the effect of gradually decreasing pH levels on the survival and glucose levels of *Pangasius* sp. The research was conducted at the Fish Breeding Research Institute from August to October 2020. The tested fish were *Pangasius* sp. seeds with a standard length of 12.31±0.84 cm, a total length of 15.25±0.98 cm and a weight of 27.29±5.44 g. Ten specimens were stocked in 30 L fiber tub per container. The study used a completely randomized design with three replications. The treatments consisted of five pH levels: A) 3.0; B) 4.0; C) 5.0; D) 6.0 and E) 7.0. This study used two testing methods: direct and gradual pH reduction. Decreasing the pH of the water was carried out by adding a 1 M solution of hydrochloric acid (HCl). The viability of the *Pangasius* sp. was calculated at 30, 60, 120, 180 and 240 minutes after testing. The blood glucose was measured at 120 minutes after testing, while the gill tissue histology was performed at the end of the test. The results showed that the difference in the pH value of the maintenance media had a significant effect ($P<0.05$) on the survival, blood glucose levels and on the level of damage to the gill tissue of the *Pangasius* sp. fry. The method of gradually decreasing the pH was able to increase the resistance of *Pangasius* sp. to acidic pH stress compared to the direct pH reduction method.
Key Words: acid water, survival level, blood glucose, gill tissue, pH stress.

Introduction. Striped catfish is a freshwater fish belonging to the *Pangasidae* family. The production of catfish in Southeast Asia in recent years has continued to increase significantly. In 2015, world catfish production reached 2.1 million tons, dominated by Vietnam with 1.1 million tons, while Indonesia and Bangladesh reached 400,000 tons (FAO 2017; Subasinghe 2017). Indonesia, as the third largest producer in the world, has catfish cultivation centers spreading across several regions. The largest catfish cultures in Indonesia are located in the island of Sumatra at 68.07%, followed by Kalimantan 20.23%, Java 8.48%, the others being scattered in various locations (KKP 2018). In the period between 2014 and 2017, there was an increase in the national catfish production by 28.91% year^{-1} (KKP 2018). Indonesia continued to increase its production through an intensification and extensification of the cultivation. The cultivation intensification program is carried out by utilizing the latest technology and using superior seeds. Meanwhile, the cultivation extensification program is carried out by optimally using land areas as catfish cultivation ponds.

Sumatra and Kalimantan, as the largest contributors to the national catfish production, having most of their peat swampland areas still sub-optimally utilized. One of the special characteristics of peatland waters is that they have an extreme pH value (Ahmad & Samat 2015). The pH value of peat swamp waters tends to be acidic and always fluctuates due to unbalanced natural conditions (Arafad 2000; Kwong et al 2014). The pH value of peatland waters ranges from 4.0 to 6.3 and in extreme conditions it can reach a pH of 3.2 (Subagyo 2006; Fatah et al 2010). Efforts should be made to optimize peatlands for catfish cultivation to support an increased national production.

Pangasius sp. has been widely cultivated in Indonesia, due to the superior seeds resulting from the hybridization between the female Siamese catfish (*Pangasianodon hypophthalmus*) and the male jambal catfish (*Pangasius jambal*) (LRPTBPAT 2006). The advantages of *Pangasius* sp. include a premium meat quality, a faster growth and the continuous availability of seeds (Tahapari et al 2016; Said & Sadi 2019). However, information regarding the physiological response and survival rate of the *Pangasius* sp. kept in acidic pH waters is not available.

Previous studies on physiological responses and survival rates on fish reared at different pH levels have been carried out on African catfish (Uzoka et al 2012; Ndubuisi et al 2015; Marimuthu et al 2019), carp (Korwin-Kossakowski 1988; Heydarnejad 2012; Prakoso & Radona 2018), flatfish (Mota et al 2017), tilapia (El-Sherif & El-Feky 2009; Reboucas et al 2015), channa (Nisa et al 2013), yellowtail kingfish (Abbink et al 2012), silver catfish (Copatti et al 2011), brook trout (Wesner et al 2011), channel catfish (Murad & Boyd 1991) and several other freshwater fish species (Baldisserotto 2011). The existence of extreme pH in rearing water causes stress in fish, as indicated by the increasing blood glucose levels, disrupting both the ionic balance in the blood and the gill tissue growth, ultimately impacting the survival of aquatic organisms (Boyd 1990; Keinanen et al 2003; Kucukgul & Sahan 2008; Marimuthu et al 2019).

Physiological changes are an indicator of an organism's ability to adapt and survive in its environment. Trials were performed under changing environmental parameters and demonstrated an increase in the adaptability of fish. Research on the adaptability in increasing salinity has been carried out in studies on *Rutilus caspicus*, tilapia and goldfish (Malakpour et al 2012; Salim et al 2016; Al Hilali & Al-Khshali 2019). Research on the adaptability to gradual changes in temperature has been carried out on tilapia, *Corogenus maraena* and *Notothenia rossii* fish (Musa et al 2017; Rebl et al 2018; Guillen et al 2019). Meanwhile, research on the adaptability to gradual changes in pH has also been carried out on goldfish and snakehead fish (Van Dijk et al 1993; Nisa et al 2013). The purpose of this study was to determine the physiological response and survival of *Pangasius* sp. that has gradually been given acidic pH stress.

Material and Method

The study was conducted at the Research Institute for Fish Breeding (RIFB), West Java, Indonesia from August to October 2020. The RIFB is one of the research centers of the Ministry of Marine Affairs and Fisheries, of the Republic of Indonesia. The research does not require ethical approval from any institution and there are no applicable laws in Indonesia.

Experimental fish. The experimental fish was the *Pangasius* sp. seed with a standard length of 12.31±0.84 cm, a total length of 15.25±0.98 cm and a weight of 27.29±5.44 g. The test fish were reared in 30 fiber tanks with a capacity of 30 L. Each tank was stocked with 10 fish, first adapted in the test container for 7 days of rearing.

pH treatment. The study used a completely randomized design with three replications. The treatments consist of five pH levels, namely: A) 3.0; B) 4.0; C) 5.0; D) 6.0 and E) 7.0 (Nisa et al 2013). Two test methods were applied, namely the direct and the gradual pH reduction. PH measurement used a Lutron pH meter PH-201. Decreasing pH was carried out by adding a 1 M solution of hydrochloric acid (HCl) to reach the desired pH level (Van Dijk et al 1993; Duan et al 2019). In the stepwise pH reduction method, the pH of the water was gradually reduced by 0.5 day^{-1} (24 hours). This method refers to the research results of Supito et al (2017). The measurement and adjustment of water pH was carried out every 12 hours to maintain a stable pH level, according to the treatment.

In the direct test, the number of dead fish in each test container was counted at 30, 60, 120, 180 and 240 minutes after treatment. As for the gradual decrease in pH, the number of dead fish was counted every 24±1 hour since the pH level decreased. The survival rate was calculated based on the equation (Effendie 1979):

Survival rate of fish (%)=(final number of fish / initial number of fish)×100

Glucose levels. Blood samples were collected for measuring the glucose levels. Blood samples were taken from the caudal vein using a syringe (1 mL), from specimens that had been given heparin as an anticoagulant and that were anesthetized using phenoxyethanol at a dose of 0.3 mL L^{-1} water (Rigal et al 2008). The measurement of blood glucose levels required a glucometer (Point of Care Test/POCT).

Histology of gill tissue. The gill tissue histology analysis was carried out based on the Humason (1967) method. The gill tissue was fixed using a NBF (Neutral Buffered Formalin) solution 10% for 24 hours and then stored in 70% ethanol solution before the histology preparation process. The gill tissue histology observation was carried out using an Olympus BX 41 trinocular microscope with 400X magnification. The parameters observed in this study were the changes in gill's structure.

Water quality parameters. The temperature and dissolved oxygen were measured at the start and end of the test with a minimum-maximum thermometer (HTC-1) and a DO meter (Lutron DO-5510), respectively.

Data analysis. The data of survival rate and glucose levels were analyzed statistically using Microsoft excel 2016 and SPSS program (ver. 20.0). One-way analysis of variance (ANOVA) was chosen to measure the significance of different treatments at a 95% confidence interval. If the treatment effect was significant, the differences between the means were examined further, using the Duncan's test. The data of the gill tissue histology was presented descriptively to report the changes of the gill structure. The water physics and chemistry data were tabulated and analyzed descriptively.

Results

Direct pH reduction method. The fish survival rates were calculated at 30, 60, 120, 180 and 240 minutes after the stress of the pH-lowering treatment. The results of the calculation of the survival rate showed that there was no significant difference between the pH levels up to 120 minutes. Significant results (P<0.05) were shown from 180 minutes and 240 minutes, between the pH 3 and pH 4 treatments. The pH 5, pH 6 and pH 7 treatments showed no significant difference (P>0.05) up to 240 minutes (Table 1).

Table 1

The survival of *Pangasius* sp. under a direct pH reduction

ph	*Survival rate after exposure (minute)*					
	0	*30*	*60*	*120*	*180*	*240*
pH 3	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	95.23± 8.25 ^{a}	0.00±0.00 ^{c}	0.00±0.00 ^{c}
pH 4	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	80.95±8.25 ^{b}	61.90±29.74 ^{b}
pH 5	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	90.47±8.25 ^{a}	90.47± 8.25 ^{a}
pH 6	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}
pH 7	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100.00±0.00 ^{a}

Survival rates in columns with the same alphabet superscript showed results that were not significantly different between direct pH reduction (P> 0.05). All values are expressed as mean ± SD.

The blood glucose levels (mg dL^{-1}) of *Pangasius* sp. were measured 120 minutes after the test fish specimens were subjected to a different pH reduction stress, as shown in Figure 1. Blood glucose levels significantly (P<0.05) increased at a pH level less than 6, whereas at pH levels 6 and 7 the blood glucose levels showed insignificant changes (P>0.05). The highest blood glucose levels were seen in pH 4 and 5 treatments (Figure 1).

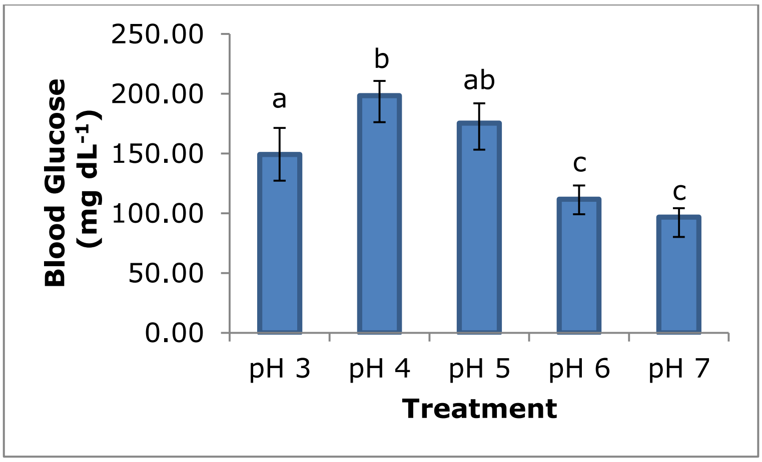

Figure 1. Blood glucose levels of *Pangasius* sp. under direct pH reduction. Blood glucose levels with the same alphabet superscript showed no significant difference between direct pH reduction (P>0.05).

Histological observations were carried out at the end of the test on the gill tissue of *Pangasius* sp., after the pH reduction stress. The results of the observation under the microscope showed differences in the structure of the gill tissue between treatments at different pH levels. Changes in the structure of the gill tissue due to a decrease in pH were directly seen in the treatment of pH 3, pH 4 and pH 5 (Figure 2).

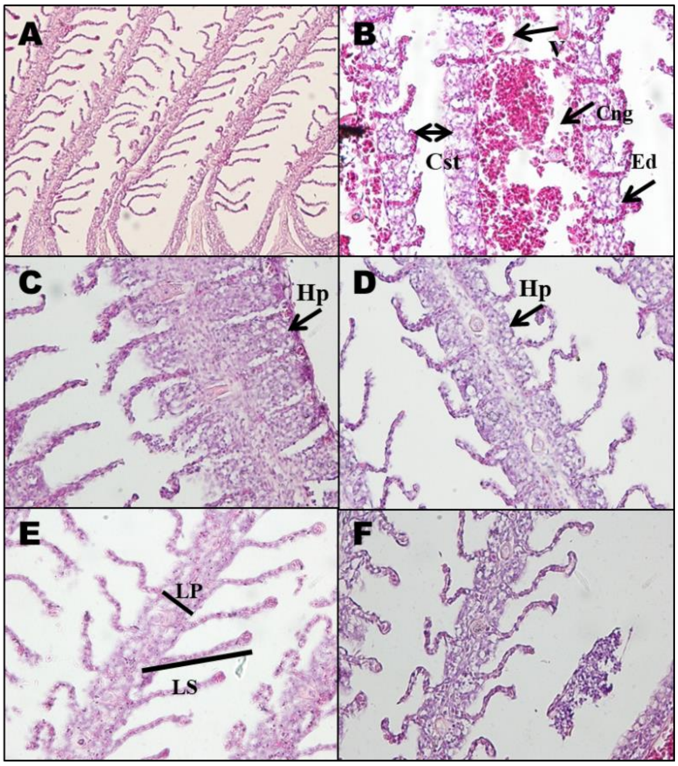

Figure 2. Histology of the gill tissue of *Pangasius* sp. after 240 minutes under the stress of direct pH reduction (A: 100x enlargement; B, C, D, E and F: 400x enlargement).

The images from Figure 2 correspond to: the control pH 7 (A and F), the treatments pH 3 (B), pH 4 (C), pH 5 (D) and pH 6 (E). The observed alterations were: hyperplasia (Hp), edema (Ed), vacuoles (V), constriction (Cst) and congestion (Cng). The gill structures such as primary lamella (LP) and secondary lamella (LS) are shown in Figure E.

Dissolved oxygen concentrations (mg L^{-1}) and temperature (°C) in the media measured during the pH reduction stress test are directly presented in Table 2. Dissolved oxygen concentration and relative temperature did not significantly change during the different pH level treatments.

Table 2
Temperature range and dissolved oxygen content of *Pangasius* sp. culture media under a direct pH reduction

Treatment	*Temperature (°C)*	*Dissolved oxygen (mg L^{-1})*
pH 3	27.8-28.1	2.2-3.1
pH 4	27.7-27.8	2.2-2.7
pH 5	27.8-27.9	2.3-2.5
pH 6	27.7-27.9	2.4-2.6
pH 7 (control)	27.7-28.0	1.9-2.5

Direct pH reduction method. The results of the calculation of the survival rate of *Pangasius* sp., which were gradually subjected to a different pH reduction stress, showed no significant difference between the pH levels, up to 120 minutes. Significant results (P<0.05) were shown only in pH 3 treatment from minutes 120, 180 and 240, while the treatment of pH 4, pH 5, pH 6 and pH 7 showed no significant difference (P>0.05) until the end of the study (Table 3).

Table 3
The survival of *Pangasius* sp. under gradual pH reduction

ph	*Survival rate after exposure (minute)*					
	0	*30*	*60*	*120*	*180*	*240*
pH 3	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}	80,95± 8,25 ^{b}	0,00± 0,00 ^{b}
pH 4	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}	95,24± 8,25 ^{a}
pH 5	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}
pH 6	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}
pH 7	100.00±0.00 ^{a}	100.00±0.00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}	100,00±0,00 ^{a}

Survival rates in columns with the same alphabet superscript showed results that were not significantly different between gradual pH reduction (P> 0.05). All values are expressed as mean ± SD.

The blood glucose level (mg dL^{-1}) of *Pangasius* sp. in the stress of decreasing pH is gradually shown in Figure 3.

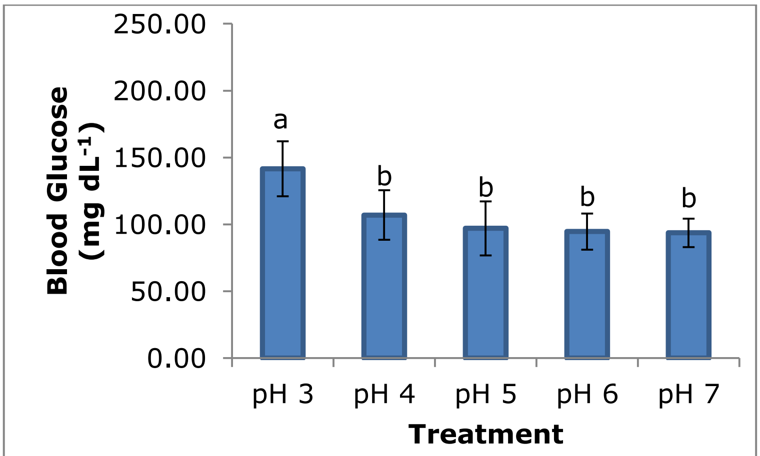

Figure 3. Blood glucose levels of *Pangasius* sp. under gradual pH reduction. Blood glucose levels with the same alphabet superscript showed no significant difference between gradual pH reduction (P>0.05).

The lower the pH of the treatment, the higher the increase of the relative blood glucose levels. However, these blood glucose levels showed insignificant changes (P>0.05) between treatments, except for the treatment at pH 3. The highest blood glucose levels were seen in treatment pH 3 and showed a significant value ($P<0.05$) with other treatment.

Histological observations were carried out at the end of the test on the gill tissue of *Pangasius* sp., after the pH reduction stress. The results of the observation under the microscope showed differences in the structure of the gill tissue between treatments of different pH levels. Changes in the structure of the gill tissue due to a decrease in pH were directly seen in the treatment of pH 3, pH 4, and pH 5 (Figure 4).

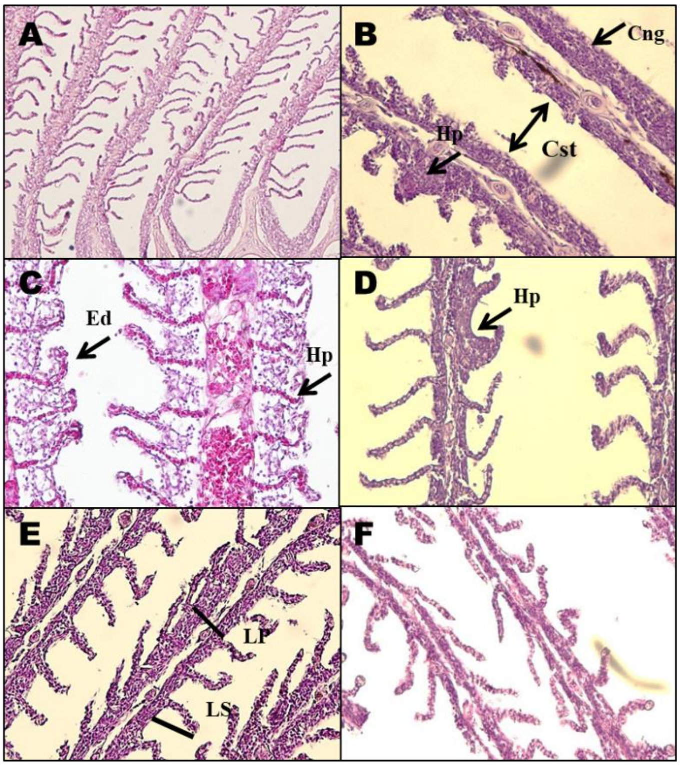

Figure 4. Histology of the gill tissue of *Pangasius* sp. after 240 minutes under the stress of direct pH reduction (A: 100x enlargement; B, C, D, E and F: 400x enlargement).

The images from Figure 4 correspond to: the control pH 7 (A and F), the treatments pH 3 (B), pH 4 (C), pH 5 (D) and pH 6 (E). The observed alterations were: hyperplasia (Hp), edema (Ed), vacuoles (V), constriction (Cst) and congestion (Cng). The gill structures such as primary lamella (LP) and secondary lamella (LS) are shown in Figure E.

Dissolved oxygen concentration (mg L^{-1}) and temperature (°C) in the media measured during the stress test to gradually decrease pH are presented in Table 4. Dissolved oxygen concentration and relative temperature did not significantly change during the treatments at different pH levels.

Table 4

Temperature range and dissolved oxygen content of *Pangasius* sp. culture media under gradual pH reduction

Treatment	*Temperature (°C)*	*Dissolved oxygen (mg L^{-1})*
pH 3	27.4-28.5	3.6-4.3
pH 4	27.3-28.0	3.5-4.3
pH 5	27.3-28.4	3.6-4.3
pH 6	27.3-28.3	3.6-4.3
pH 7 (control)	27.2-28.0	3.5-4.3

Discussion. Each aquatic organism has an optimal tolerance value and pH requirements in its environment. Freshwater fish generally requires an optimal range for water pH between 6.5 and 8.5 (Parra & Baldisserotto 2007). Water conditions with a pH that is too low or too high, compared to the threshold of fish's capacity to adapt, can cause stress, irritation and lesions on the skin, changes in fish behavior, changes in skin color, irritation of the gills, increased production of mucus, respiratory problems and death (Roberts & Palmeiro 2008; Husni & Esmiralda 2010).

In this study, *Pangasius* sp. was given environmental stress in the form of a direct and gradual decrease in pH, from normal pH (pH 7.0-8.0) to pH 3.0, 4.0, 5.0, 6.0 and 7.0 (control). *Pangasius* sp. responding to the pH decrease showed different results by the direct and gradual methods. In the direct pH reduction method, the tolerance range for acidic pH stress of *Pangasius* sp. is at a level of ≥5.0. *Pangasius* sp. was not able to survive at pH 3.0 for 180 minutes. Even at treatment of pH 4.0, the survival rate of *Pangasius* sp. at 240 minutes after testing was only 61.90±29.74% (Table 1). Meanwhile, in the gradual pH decrease method, the tolerance range for acidic pH stress of *Pangasius* sp. was at a level of ≥4.0. *Pangasius* sp. was still able to survive at a pH level of 4.0, with a survival rate of 95.24±8.25%, for 240 minutes (Table 3). Previous research stated that stickleback fish (*Gasterosteus aculeatus*) had a pH tolerance range of 4.0-5.0, cichlid fish (*Cichlasoma urophthalmus*) had a pH tolerance range of 6.5-9.2, perch fish (*Anabas testudineus*) had a pH tolerance range of 4.6-9.5, African catfish (*Clarias gariepinus*) had a pH tolerance range of 6.5-8.0, and goldfish (*Cyprinus carpio*) had a pH tolerance range of 4.0-7.0 (Ndubuisi et al 2015; Himawan et al 2016; Prakoso & Radona 2018). Deaths that occur at pH levels outside the tolerance range are due, among others, to the stress experienced by fish and to the damage of the gill tissue (Kucukgul & Sahan 2008; Roques et al 2010; Marimuthu et al 2019).

In the present study, a direct decrease in pH triggered stress for *Pangasius* sp. fish more quickly than the gradual pH reduction method. Stress in fish can be indicated by an increase in blood glucose levels (Kucukgul & Sahan 2008). When the fish are stressed, there is an increase in the distribution of glucose in the blood, which is accompanied by insulin inactivation, so that glucose cannot enter the cells (Hastuti et al 2003). Catfish blood glucose levels in optimal environmental conditions are in the range of 76.82-131.74 mg dL^{-1} (Susanto et al 2014). On direct exposure to pH, the *Pangasius* sp. experienced stress at the treatment with a pH level of ≤5, with blood glucose levels of 149.33-198.33 mg dL^{-1}. On gradual exposure to pH, on the other hand, *Pangasius* sp. experienced stress at the pH 3 treatment, with blood glucose levels of 141.67 mg dL^{-1}. In this study, the *Pangasius* sp. that did not experience stress had glucose levels ranging from 93.67 to 111.67 mg dL^{-1}.

The results of the *Pangasius* sp. gill tissue histology observations showed some changes in the structure of the gill tissue due to the stress of the decrease in pH of the rearing water. Gill tissue damage can be in the form of cell swelling (edema), hyperplasia, vacuoles, constriction and congestion (Jamin & Erlangga 2016; Pribadi et al 2017; Mansouri et al 2018). Hyperplasia was seen in stress treatment with pH ≤5.0. In the pH 5.0 treatment with the direct pH reduction method, hyperplasia was seen in almost all secondary lamellae (Figure 2D). With the stepwise pH reduction method, on the other hand, hyperplasia in pH 5.0 was only seen in some secondary lamellae of *Pangasius* sp. gills (Figure 4D). The mortality of all fish in the pH 3.0 treatment was thought to have occurred due to: edema, hyperplasia, vacuoles, constriction and congestion, which in turn could cause bleeding.

The results showed that the daily temperature and dissolved oxygen in the test media were in the optimal range and did not experience significant fluctuations. Tahapari et al (2014) stated that the optimal temperature for the maintenance of *Pangasius* sp. seeds is in the range of 25.0-31.0°C, with a dissolved oxygen content of more than 3 mg L^{-1}.

Conclusions. The difference in the pH value of the maintenance medium had a significant effect on the survival, blood glucose levels and the level of damage to the gill tissue of the *Pangasius* sp. Decreasing pH gradually by 0.5 every 24 hours can produce an increase of the resistance of *Pangasius* sp. to acidic pH stress until it reaches a pH level of 4.0. In the direct pH reduction method, on the other hand, the *Pangasius* sp. seeds were able to survive for only 240 minutes of exposure at a pH level of 5.0.

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Conflict of interest. The authors declare no conflict of interest.

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