Effects of acidic sulfate water on growth, survival, and digestive enzyme activities of striped catfish (*Pangasianodon hypophthalmus*) fingerlings

Nguyen T. K. Ha, Le H. Thang, Nguyen T. Em, Tran T. Giang, Nguyen T. Phuong, Do T. T. Huong

College of Aquaculture and Fisheries, Can Tho University, Campus 2, 3/2 street, Can Tho city, Vietnam. Corresponding author: D. T. T. Huong, dtthuong@ctu.edu.vn

Abstract. The study aimed to evaluate the effect of low pH water from acid sulfate soil on growth parameters and digestive enzyme activities of striped catfish (*Pangasianodon hypophthalmus*) fingerling stage (11.1±0.12 g). The fish was exposed to six levels of water pH including 4.5, 5.0, 5.5, 6.0, 6.5, and 7.2 (tap water as control). The treatments were randomly arranged and triplicated in 500-L fiberglass tanks with a density of 40 fish per tank for 60 days. The results showed that the average fish weights at the pH 6.0, 6.5, and 7.2 (control) treatments were significantly higher than those at the pH 4.5, 5.0, and 5.5 treatment ($p < 0.05$), and the highest was in the pH 6.5 treatment (40.1±0.22 g). The longest body length was found in the pH 6.5 treatment (17.2±0.12 cm), statistically significant in comparison with fish at the pH 4.5 treatment (14.1±0.47 cm) ($p < 0.05$). The daily weight gain (DWG) and specific growth rate (SGR) were highest at the pH 6.5 treatment compared to the other treatments. The feed conversion ratio (FCR) was highest at the pH of 6.5 (1.08) while the lowest was at the pH 4.5 (1.64). The survival rates fluctuated between 92.5 and 96.6%, but they were insignificantly different among treatments. The activities of trypsin and chymotrypsin enzymes were significantly decreased in the fish reared in the pH lower than 6.0 while pepsin and amylase enzyme activities showed no effect by the pH levels. The results suggest that the acid sulfate soil areas with slightly low pH (about 6.5) could be considered for striped catfish aquaculture.

Key Words: chymotrypsin, growth performance, low pH, trypsin.

Introduction. Acid sulfate soils have been described as the nastiest soils in the world because they contain a high amount of sulphuric acid, resulting in an acid environment with a pH reaching about 2 (Dent & Pons 1995). Aquatic organisms living in this acidic environment could be rendered stunted, sickly, or even died at lethal doses of aluminum and other toxic elements (Dent & Pons 1995). In Vietnam, it was reported that the area of acid sulfate soils was 1.8 million hectares accounting for 5.5% of the country's total area. About 1.6 million hectares of this land were situated in the Mekong delta region with pH as low as 3.5 (Khang et al 1998; MARD 2017). In the acid sulfate soil regions, the decrease in soil and water pH interferes with its aquaculture production because low pH impacts the growth of aquatic life in either direct or indirect ways. Impacts on aquatic life can be seen after rain events, especially following a period of drier conditions, flushing a large amount of sulfuric acid and toxic, heavy metals into water associated with acid sulfate soil, resulting in fish kills (White et al 1997), diseases as an outbreak of epizootic ulcerative syndrome, reproduction, recruitment, and growth problems for cultured species (Sammut et al 1995). Teleost fish showed a higher growth rate at the pH between 6.5 and 9.0, and fish growth interfered as pH was below 6.0 (Zweig et al 1999). Some studies proved that low water pH influences the growth performance and feed consumption of some cultured species (Abbink et al 2012; Kennedy & Picard 2012). Most of the fish species are able to tolerate both rather low pH (4.0-5.0) and very high pH (9.0-10.0); the mortalities, however, have been found in a few hours when fish exposed to pH out of this range (Parra & Baldisserotto 2007). The larval weight and length of silver catfish (*Rhamdia quelen*) was rapidly decreased when being nursed at pH

6.0 and 7.0 compared to higher pH levels and larvae being died at pH 5.5 after 21 days of exposure (Lopes et al 2001). Another trial on *R. quelen* grown at pH 5.2-6.5 presents lower growth compared to fish cultured at pH 7.5 (Copatti et al 2005). Atlantic salmon cultured at pH 4.2-5.0 showed lower growth than those grown at pH 5.2-6.5 (Norrgren & Degerman 1992). Most recently study by Darmawan et al (2021) indicated that catfish (*Pangasius* sp.) could not survive at pH less than 3.0 with a decreased survival rate at pH 4.0. In addition, it was also found that reduced pH increased the plasma cortisol concentration causing non-specific immunity of fish (Brown et al 1990).

Pangasianodon hypophthalmus was considered as the important cultured species in Vietnam, particularly the Mekong delta accounting for the largest aquaculture area and highest production with 5.700 hectares and 1.56 million tons in 2020 (VASEP 2020). Previous studies in *P. hypophthalmus* mostly focused on environmental effects such as salinity (Lam et al 2011; Huong & Quyen 2012; Phuc et al 2014; Ha et al 2021), temperature (Phuc 2015; Huong et al 2020), dissolved oxygen (Phuong et al 2017) or nitrite (Lefevre et al 2011) on fish physiology as well as growth performance. The effects of pH, especially low water pH caused by drainage from acid sulfate soils on *P. hypophthalmus* have not attracted much attention from scientists although the areas of acid sulfate soils have been increasing year by year by reclamation, especially to grow rice (Minh & Vu 2015). Therefore, the purpose of this study is to elucidate the influences of low water pH formed by acid sulfate soils on growth performance, survival rate, and enzymatic activities of *P. hypophthalmus*. The gained results could contribute to the extension of *P. hypophthalmus* aquaculture in the acid sulfate soil region, such as in the Mekong delta of Vietnam.

Material and Method

Experimental material. The study was conducted at the College of Aquaculture and Fisheries, Can Tho University from April 2021 to June 2021. Seven hundred twenty *P. hypophthalmus* fingerlings (11.1±0.12 g) were artificially produced at the College of Aquaculture and Fisheries, Can Tho University. Acid sulfate soils were obtained from Hoa An village, Phung Hiep district, Hau Giang province, Vietnam. The soils (wet form) were then mixed with tap water (pH 7.21) with the portion of 50% water : 50% soil (v/v) for 3 days, resulting in highly acidic water with a pH of around 2.5-3.0. This low pH water was then diluted with tap water to obtain different experimental pH levels.

Experimental design. There were six pH levels representing different experimental treatments including 4.5, 5.0, 5.5, 6.0, 6.5, and 7.21 (using tap water as control) with three replicates for each. *P. hypophthalmus* fingerlings were randomly distributed into 18 fiberglass tanks (500-L) (containing 300-L of water) with a density of 40 individuals per tank. After three days, water pH in the tanks was decreased in a stepwise fashion by 0.25 units every 2 hours, monitored by a pH meter (Hanna model HI98128). Treatments with lower pH levels were adjusted before the higher pH treatments in order to get all the treatments to reach the target pH levels at the same time. The experimental period was 60 days.

The water in the culture tanks was continuously supplied with oxygen by aeration throughout the experimental period. Besides, pH was controlled according to the target levels by frequently checking pH values in every single tank (three times/day). Highly acidic water or tap water was added into the tanks as pH moved out of the experimental levels. Fish were fed to satisfaction twice a day with commercial pellets (7434 - Cargill, 2 mm-35% protein), and uneaten pellets were removed about 30 minutes post-feeding. Water exchange of 50% was conducted weekly. The number of dead fish was recorded for determining the survival rate.

Water quality parameters. The water quality parameters such as pH, temperature, and dissolved oxygen (DO) were checked twice a day. NO_2^-, total ammonia nitrogen (TAN), and SO_4^{2-} were measured once a week. Total iron (Fe) and aluminum (Al) were measured at the beginning and the end of the experiment. Concentrations of NO_2^- and

TAN were measured using the methods of Griess llosvay and Diazonium and Indophenol blue, respectively. The concentration of SO_4^{2-} was analyzed using a method described by APHA (2012). Total Fe and Al were measured using the method of APHA (1995).

Growth parameters. The initial weight and length of fish were measured at the beginning of the experiment. On day 30th and 60th, the number of fish in each tank was counted and fish weight and length were determined by measuring all the fish in each tank.

$$\text{Survival rate (SR, \%)} = \frac{\text{Number of fish at the end of the experiment}}{\text{Number of fish at the beginning of the experiment}} \times 100$$

$$\text{Daily weight gain (DWG, g day}^{-1}\text{)} = \frac{Wt - W0}{t}$$

$$\text{Specific growth rate (SGR, \% day}^{-1}\text{)} = \frac{Ln(Wt) - Ln(W0)}{t} \times 100$$

$$\text{Daily length gain (DLG, cm day}^{-1}\text{)} = \frac{Lt - L0}{t}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake}}{\text{Fish weight gain}}$$

where: W_0, L_0: initial weight (g) and initial total length (cm) respectively; Wt, Lt: final weight (g) and final total length (cm) respectively; t: experimental time (day).

Digestive enzymatic activities. At the end of the experiment, feeding was ceased for one day before sampling the stomach and anterior part of the fish gut to analyze digestive enzymatic activities (pepsin in the stomach; amylase, trypsin, and chymotrypsin in the gut). The samples of stomach and gut were homogenated with the buffer KH_2PO_4 20 mM and NaCl 6 mM, pH 6.9. The mixture was then centrifuged at 4,200 × g for 30 minutes at 4^{o}C. The supernatant was collected and stored at -80°C for further analyses. Chymotrypsin and pepsin activities were measured by the method described by Worthington (1982); trypsin was measured by the method of Tseng et al (1982); amylase was measured by the method of Bernfeld (1951). Specific activities were analyzed using the method of Bradford (1976) and expressed as U mg protein^{-1} min^{-1}.

Statistical analysis. All the data were subjected to statistical treatment involving standard deviation (SD) and mean (M) using Excel 2016. One-way analysis of variance (ANOVA) together with DUNCAN's post-hoc tests were used to test for significant differences among treatments (at a significant level of $p < 0.05$) by using SPSS 18.0.

Results

Water parameters. Throughout the experimental period, water pH in different treatments was maintained around the set levels with values of 4.54±0.09; 5.03±0.11; 5.52±0.09; 6.01±0.08; 6.46±0.09; 7.21±0.20 at the treatments of 4.5, 5.0, 5.5, 6.0, 6.5, and 7.2, respectively. The temperature ranged between 28.4 and 28.6°C, while DO remained at high concentrations of 6.71-7.03 mg L^{-1} (Table 1). NO_2^- concentrations ranging between 0.008 and 0.186 mg L^{-1} reveal increases with the pH levels. TAN and SO_4^{2-} concentrations increased in low pH treatment compared to the higher pH treatments, which fluctuated from 0.16 to 2.14 and from 1.09 to 13.5 mg L^{-1}, respectively. The concentrations of Al and Fe were decreased as the pH levels increased, with values ranging from 0.318 to 0.023 mg L^{-1} for Al and from 0.094 to 0.021 mg L^{-1} for Fe (Table 2).

Table 1
The values of pH, temperature, DO, TAN and NO_2^- in experimental tanks during the experiment

pH treatments	*pH*	*Temperature (°C)*	*DO ($mg\ L^{-1}$)*	*TAN ($mg\ L^{-1}$)*	*NO_2^- ($mg\ L^{-1}$)*
4.5	4.54±0.09	28.4±1.34	7.03±0.02	2.14±0.12	0.008±0.019
5.0	5.03±0.11	28.4±1.36	6.98±0.02	2.19±0.09	0.034±0.058
5.5	5.52±0.09	28.6±1.31	7.00±0.03	2.20±0.05	0.027±0.022
6.0	6.01±0.08	28.5±1.30	6.80±0.06	2.20±0.08	0.067±0.060
6.5	6.46±0.09	28.4±1.44	6.71±0.01	0.51±0.19	0.186±0.143
Control	7.21±0.20	28.5±1.28	6.95±0.00	0.16±0.10	0.154±0.070

Values are presented as mean±SD.

Table 2
The concentration of SO_4^{2-}, Al and Fe in experimental tanks during the experiment

pH treatments	*SO_4^{2-} ($mg\ L^{-1}$)*	*Al ($mg\ L^{-1}$)*	*Fe ($mg\ L^{-1}$)*
4.5	13.5±0.67	0.318±0.186	0.094±0.079
5.0	9.13±2.83	0.193±0.033	0.083±0.026
5.5	7.97±0.63	0.117±0.040	0.062±0.033
6.0	7.99±0.06	0.032±0.007	0.051±0.022
6.5	2.24±0.24	0.031±0.005	0.026±0.004
Control	1.09±0.37	0.023±0.002	0.021±0.015

Values are presented as mean±SD.

Survival rate*.* The results showed high tolerance of *P. hypophthalmus* to a low pH environment, which is not a significant difference among the treatments. The survival rates of fish were also somewhat high, ranging between 92.5 and 96.7%. The lowest survival rates were at the pH 5.0 and 5.5 treatments (92.5%) and the highest was at pH 4.5 treatment (96.6%).

Growth performance*.* After 30 days of culture, the average fish weight at the pH 6.5 treatment presented the highest increase (23.7±0.19 g), which was rapidly higher than the other treatments ($p < 0.05$). The growth performance of fish decreases with a decrease of the pH treatments from 6.5 to 4.5, with only one exception visible for the pH 6.0 treatment. The lowest average body weight (14.8±1.43 g) was found in the pH 4.5 treatment. On day 60th, the average body weight of fish in different treatments followed the same pattern as seen on the day 30th; fish in the pH 6.5 treatment showed the highest weight (40.1±0.22 g) and lowest at the pH 4.5 treatment with values of 20.3± 1.90 g ($p < 0.05$) (Figure 1).

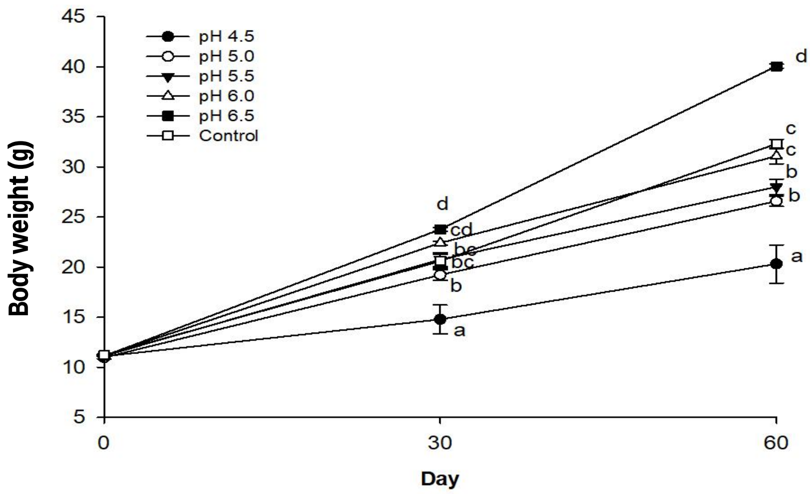

Figure 1. The average body weight of *P. hypophthalmus* cultured in different pH levels. Lines with different letters (a, b, c, d) signify a significant difference ($p < 0.05$) at the same sampling time.

On the other hand, the increase in fish length followed the same trend as seen in the growth in fish weight. The lengths of fish in the pH 6.0 and 6.5 treatments after 30 days were significantly higher than the other treatments ($p < 0.05$). On day 60th, the highest body length was likely observed for the pH 6.5 treatment (17.2±0.12 cm), being rapidly higher than those in the other treatments ($p < 0.05$) (Figure 2).

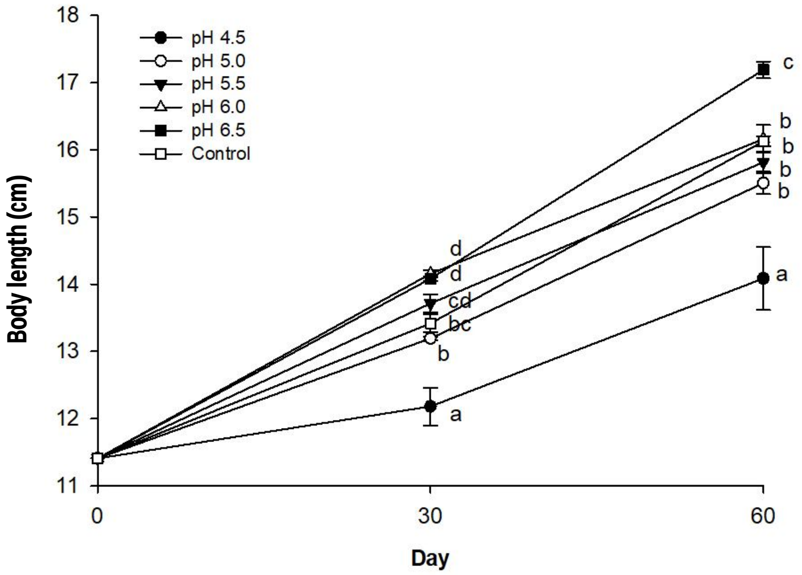

Figure 2. The average body length of *P. hypophthalmus* cultured in different pH levels. Lines with different letters (a, b, c, d) signify a significant difference ($p < 0.05$) at the same sampling time.

Daily weight gain and specific growth rate. The same trend as seen in the results of body weight and length was repeated in DWG and SGR. After 60 days, DWG and SGR were highest at pH 6.5 with values of 0.482±0.004 g day^{-1} and 6.047±0.009% day^{-1}, respectively, and these growth indicators decreased with the decrease in pH. The lower the pH was, the lower the DWG and SGR recorded with the exception being visible in the control treatment. It is also noted that DWG and SGR in different pH treatments were significantly different ($p < 0.05$). Similarly, DLG was likewise highest at pH 6.5 (0.096± 0.002 cm day^{-1}), which was significantly higher than those in the other treatments (Table 3).

Table 3

Daily weight gain, specific growth rate and daily length gain of *P. hypophthalmus* cultured in different pH levels for 60 days

pH treatments	*DWG (g day^{-1})*	*SGR (% day^{-1})*	*DLG (cm day^{-1})*
4.5	0.154±0.032^{a}	4.790±0.183^{a}	0.045±0.008^{a}
5.0	0.260±0.007^{b}	5.309±0.035^{b}	0.068±0.003^{b}
5.5	0.281±0.013^{b}	5.403±0.050^{bc}	0.073±0.003^{b}
6.0	0.333±0.013^{c}	5.592±0.044^{c}	0.079±0.003^{b}
6.5	0.482±0.004^{d}	6.047±0.010^{d}	0.096±0.002^{c}
Control	0.351±0.007^{c}	5.662±0.024^{c}	0.079±0.001^{b}

Values are presented as mean±SE; different subscript letters (a, b, c) in the same column indicate significant differences ($p < 0.05$).

Feed conversion ratio. The result indicated that low pH conditions affected the FCR of fish in different pH treatments. The highest value was noted at the pH 4.5 treatment (1.64) which was significantly higher than other treatments, while the lowest FCR was found at the pH 6.5 treatment (1.08), not being dramatically different from the pH treatments of 5.0, 5.5, 6.0, and control (Figure 3).

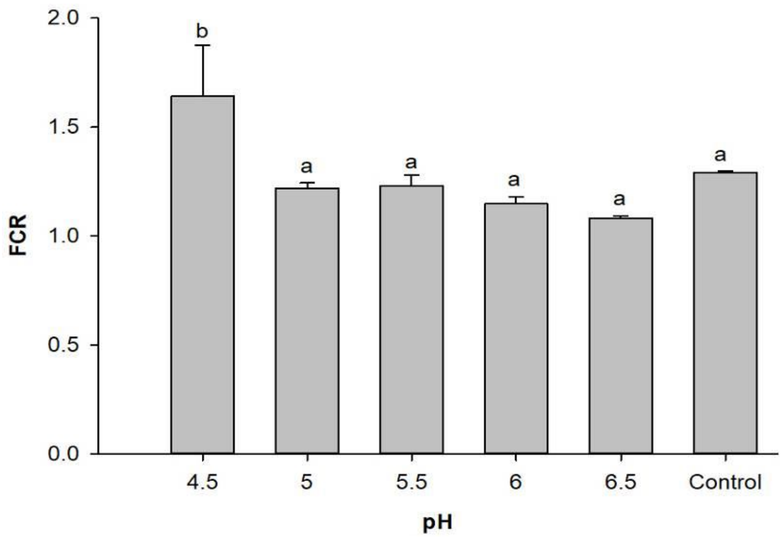

Figure 3. The FCR of *P. hypophthalmus* cultured in different pH levels. The bars with different letters (a, b) signify a significant difference ($p < 0.05$).

Digestive enzyme activities. After 60 days of culture in different pH conditions, the results of the enzymatic analysis showed an increase in trypsin and chymotrypsin activities when pH increases from 4.5 to 6.5 followed by a decrease in the control treatment. The highest activities of trypsin and chymotrypsin were found at the pH 6.5 treatment (0.0075±0.00094 and 294±30.8 U min^{-1} mg $protein^{-1}$), which were significantly higher ($p < 0.05$) than those at the pH 4.5 and 5.0 treatments with values of 0.0049±0.00041; 0.0056±0.00039 and 173±24.5; 178±18.9 U min^{-1} mg $protein^{-1}$, respectively. It also revealed that the enzymatic activities from pH 5.5 to 6.5 and control treatments were not significantly different. On the contrary, the other two types of enzymes were not impacted by different pH levels ($p > 0.05$) (Table 4).

Table 4

Digestive enzyme activities of *P. hypophthalmus* cultured in different pH levels

pH treatment	Enzymes (*U min^{-1} mg $protein^{-1}$*)			
	Trypsin	*Chymotrypsin*	*Amylase*	*Pepsin*
4.5	0.0049±0.00041[a]	173±24.5[a]	0.69±0.10[a]	0.32±0.02[a]
5.0	0.0056±0.00039[ab]	178±18.9[a]	0.64±0.02[a]	0.42±0.10[a]
5.5	0.0060±0.00061[abc]	254±21.2[ab]	0.63±0.02[a]	0.42±0.01[a]
6.0	0.0060±0.00006[abc]	284±38.3[b]	0.64±0.01[a]	0.40±0.03[a]
6.5	0.0075±0.00094[c]	294±30.8[b]	0.71±0.04[a]	0.40±0.08[a]
Control	0.0071±0.00022[bc]	234±14.6[ab]	0.64±0.03[a]	0.38±0.07[a]

Values are presented as mean±SE; different subscript letters (a, b, c) in the same column indicate significant differences ($p < 0.05$).

Discussion

Water parameters. The variations in water temperature and DO during the experiment were within a normal range for fish (Boyd 1998). Changing pH values could change the toxic level of chemical compounds in water. The addition of sulfate water to maintain the target pH levels impacted the TAN and SO_4^{2-} concentrations. Specifically, TAN and SO_4^{2-} concentrations increased in low pH treatments compared to the higher pH treatments. This could be explained that the initial concentrations of TAN and SO_4^{2-} were high and decreased when being dissolved with tap water. This explains the high TAN and SO_4^{2-} concentrations in low pH treatments where the dilution ratio with tap water is lower than those in high pH treatments. This can be most prominently seen in the control (tap water) treatment with no acidic sulfate water being used. NO_2^- concentrations increased with the pH levels during the experiment. Al and Fe concentrations follow the same pattern as seen in the result of SO_4^{2-} concentration analysis, and the same reason could be used to explain the decreasing trend of Al and Fe concentrations when pH levels increase. Boyd (1998) suggested the suitable limits of SO_4^{2-} and Fe for aquatic organisms which are 5-100 mg L^{-1} and 0.05-0.5 mg L^{-1}, respectively. The negative effects on aquatic biota and particularly the high toxic concentrations of dissolved Al (also partly complexed with sulfate) can be expected to have acute effects on fish and other organisms (Nystrand & Österholm 2013). It has been stated that a dissolved Al concentration of around 0.5 mg L^{-1} will generally eliminate most aquatic species in acidic waters (Earle & Callaghan 1998). Additionally, Gostomski (1990) said that the average concentration of Al does not exceed 0.75 mg L^{-1} when the ambient pH is between 6.5 and 9.0 to protect from acute toxicity. On the contrary, *Morone saxatilis* and *Salvelinus fontinalis* exposed to an Al concentration of 87 mg L^{-1} neither cause mortality nor significant weight loss. In the current study, Al concentrations at the 4.5 and 5.5 treatments exceeded the suitable range for aquatic organisms, which may be toxic to fish. Generally, these water quality parameters (except Al) during the experiment are within the acceptable limits for aquatic species.

***Survival rate*.** The resistance of fish to unfavorable environments, particularly in low pH conditions is species-specific. The present study reveals high survival rates being from 92.5 to 96.6%, implying that *P. hypophthalmus* is capable to survive in acidic sulfate soil drainage affected water conditions with pH reaching as low as 4.5 for a long period. Daily observation noted some fish with slow swimming, loss of appetite, and decreases in fish weight compared to the initial weight. These fish died either a few days after exposure or after day 30th, explaining the decreased survival rates of fish in the experiment. The tolerance of the *P. hypophthalmus* to low pH conditions was previously highlighted by Yen (2003), where larvae (0.74-0.77 g) can survive at a pH value of 3.79±0.1. As stated by Darmawan et al (2021), who investigated the effects of decreasing pH (using HCl) by both direct and gradual pH reduction methods on the survival rate of *Pangasius* sp., fish were not able to survive at pH 3.0 in the direct pH reduction method, and the survival rates of 61.9±29.7% and 90.5±8.25% were recorded at pH levels of 4.0 and 5.0, respectively. At pH of 6.0 and 7.0, no mortality was recorded after 240 minutes of exposure. Oppositely, the survival rates were higher when using the gradual pH reduction method, being 95.2±8.25% at the pH 4.0 and 100% at pH higher than 5.0. In this study, pH was reduced by 0.25 units every 2 hours and the fish survival rate was highest at the pH 4.5 which showed the pH reduction method did not affect the survival of fish.

***Growth performance*.** *P. hypophthalmus* fingerlings reared in low pH water for a long period show great influences on their growth performance. The results, as aforementioned, indicate that fish weight decreased as pH was reduced lower than 6.0. There is only a limited number of published papers about the effects of acidic sulfate soil drainage on the striped fish as well as other aquatic species. The studies of the effect of acid revealed that the low pH condition has impacts on the physiology and growth of cultured fish. As stated by Wood (2001), high H^+ concentrations in acidic water disrupt the tight junctions of gill epithelia, increasing ion loss via paracellular routes. This results in changes in plasma ion levels, subsequently requiring more energy to maintain ionoregulatory balance. This explains the lower growth observed in *P. hypophthalmus* reared at low pH (4.5-6.0) in this study. The loss of plasma ions (Na^+) was also found in *R. quelen* fingerlings exposed to pH 5.0 and 6.0 compared to 7.5 (Bolner & Baldisserotto 2007). A decrease in ion Na^+ concentration was also observed in *Perca flavescens* (Freda & McDonald 1988). Ha et al (2017) also demonstrated that *Pangasius bocourti* exposed to pH 6.39±0.05 (high CO_2 level) showed decreases in ion Na^+ and Cl^- concentrations, and plasma osmolality and growth performance. Previous research on *R. quelen* showed that larvae exposed to pH 5.5 and 6.0 had lower length and weight than larvae exposed to higher pH (Lopes et al 2001). Besides, some studied species presented a better growth in slightly acidic water (Alabaster & Lloyd 1982). The explanation given by the authors is due to the reduced competition for food. In a study to investigate the influences of acetic acid and sodium carbonate on *Trichogaster lalius*, Sahu & Datta (2018) reported lower weight gain and specific growth rate at pH levels of 5.0, 6.0, and 9.0 compared to either pH 7.0 or 8.0. Another similar result was published by Menendez (1976) on *S. fontinalis* fingerlings where the fish growth at water pH 5.5, 6.0, and 6.5 was lower than those in pH 7.1. This finding was supported by Ndubuisi et al (2015), where pH 7.0 was proven to have a better growth of *Clarias gariepinus* fry when they were exposed to at 11 pH levels (2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) for 14 days. A decrease in length and weight of *Lepomis macrochirus* was also recorded at pH 5.5 compared to pH 6.5 and 7.5 (Brogowski et al 2005).

Digestive enzymatic activities. Acidic sulfate water does not only directly affect the growth of *P. hypophthalmus*, but also its digestive enzymatic activities. In the present study, trypsin and chymotrypsin activities decreased in fish cultured at low pH conditions. Trypsin and chymotrypsin play an important digestive enzyme in fish, hydrolyzing peptide bonds of various amino acids including lysine, arginine, and aromatic amino acids (Huong & Tu 2010). Additionally, trypsin is considered a key proteolytic and self-activated enzyme influencing the activity of other pancreatic zymogens; it potentially limits the growth rate of *Gadus morhua* (Lemieux et al 1999). The decrease in trypsin and chymotrypsin enzyme activities is likely to result in decreases in the absorption of protein in consumed food and the growth performance of fish. In addition, the disruption in the intestinal morphology was found by Ibrahim et al (2020) who reared *Cyprinus carpio* in different acidic conditions with pH of 6.6, 5.8, and 5.0. The authors also noted the formation of necrosis and atrophy of intestinal villi, submucosal edema, and mucosal necrosis and the massive destruction of the mucosal epithelium in fish under various acidic stress. This disruption possibly reduces the amount of secreted enzymes and consequently impairment of digestive and absorptive processes and their physiological efficiencies. The deficiencies in digestion and absorption of food influence the FCR of *P. hypophthalmus*. In highly acidic sulfate water (pH 4.5), the FCR was significantly higher than other pH levels. On the other hand, low pH condition stresses the *Pangasius* sp., leading to increase in blood glucose concentration and damage of gills (Darmawan et al 2021). Fish use more energy to prevent the negative impacts of low pH rather than growth, resulting in a decrease in growth and an increase in FCR. The effects such as reduction in growth, appetite and feed conversion efficiency, and disruption to physiological homeostasis of low pH were reported to be more severe than at higher pH (Abbink et al 2012).

Conclusions. *P. hypophthalmus* can survive under highly acidic sulfate water with pH reaching as low as 4.5, but their growth is under the great influence. The pH lower than 6.0 causes a decrease in fish growth and digestive enzyme activities, with an increase in FCR. Generally, slightly acidic sulfate water with a pH of 6.5 could be considered for *P. hypophthalmus* aquaculture. In addition, it is interesting that fish growth indicators are greater in the pH 6.5 if compared to those of control pH 7.2 so further investigation on this would be necessary.

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Conflict of interest. The authors declare that there is no conflict of interest.

References

Abbink W., Garcia A. B., Roques J. A., Partridge G. J., Kloet K., Schneider O., 2012 The effect of temperature and pH on the growth and physiological response of juvenile yellowtail kingfish *Seriola lalandi* in recirculating aquaculture systems. Aquaculture 330:130-135.

Alabaster J. S., Lloyd R., 1982 Water quality criteria for freshwater fish. 2nd edition. Food and Agriculture Organization of the United Nations, Butterworths, Cambridge, 361 pp.

APHA, 2012 Standard methods for the examination of water and wastewater. 22nd edition. American Public Health Association, Washington DC.

APHA, AWWA, WEF, 1995 Standard methods for the examination of water and wastewater. 19th edition. American Public Health Association, Washington DC.

Bernfeld P., 1951 Enzymes of starch degradation and synthesis. Advances in Enzymology and Related Areas of Molecular Biology 12:379-428.

Bolner K. C. S., Baldisserotto B., 2007 Water pH and urinary excretion in silver catfish *Rhamdia quelen*. Journal of Fish Biology 70(1):50-64.

Boyd C. E., 1998 Water quality management for pond fish culture: research and development. International Center for Aquaculture and Aquatic Environments, v.43, pp. 1-37.

Bradford M. M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2):248-254.

Brogowski Z., Siewert H., Keplinger D., 2005 Feeding and growth responses of bluegill fish (*Lepomis macrochirus*) at various pH levels. Polish Journal of Environmental Studies 14:517-519.

Brown S. B., MacLatchy D., Hara T. J., Eales J. G., 1990 Effects of low ambient pH and aluminum on plasma kinetics of cortisol, T3, and T4 in rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Zoology 68:1537-1543.

Copatti C. E., Coldebella I. J., Radünz Neto J., Garcia L. O., Da Rocha M. C., Baldisserotto B., 2005 Effect of dietary calcium on growth and survival of silver catfish fingerlings, *Rhamdia quelen* (Heptapteridae), exposed to different water pH. Aquaculture Nutrition 11(5):345-350.

Darmawan J., Pribadi T. D., Haryadi J., 2021 The effect of gradual decrease in pH level on the survival rate and glucose levels of catfish (*Pangasius* sp.). AACL Bioflux 14(3):1231-1241.

Dent D. L., Pons L. J., 1995 A world perspective on acid sulfate soils. Geoderma 67(3-4):263-276.

Earle J., Callaghan T., 1998 Effects of mine drainage on aquatic life, water uses, and man-made structures. In: Coal mine drainage prediction and pollution prevention in Pennsylvania. Keith B. C. B., Timothy K., Michael W. S., Roger J. H. (eds), Pennsylvania Department of Environmental Protection, Harrisburg, pp. 4-1 to 4-10.

Freda J., McDonald D. G., 1988 Physiological correlates of interspecific variation in acid tolerance in fish. Journal of Experimental Biology 136:243-258.

Gostomski F., 1990 The toxicity of aluminum to aquatic species in the US. Environmental Geochemistry and Health 12(1):51-54.

Ha N. T. K, Bieu N. T. X, Phuong N. T., Huong D. T. T., 2017 Effect of CO_2 on acid-base regulation and growth performance of basa catfish (*Pangasius bocourti*). Can Tho University Journal of Science 54(2):18-26.

Ha N. T. K., Em N. T, Ngoc N. M, Takagi Y., Phuong N. T, Huong D. T. T., 2021 Effects of salinity on growth performance, survival rate, digestive enzyme activities and physiological parameters of striped catfish (*Pangasianodon hypophthalmus*) at larval stage. Can Tho University Journal of Science, Special issue: Aquaculture and Fisheries (13):1-9.

Huong D. T. T., Tu N. V., 2010 [Introduction to the physiology and fish and crustacean]. Agricultural publishing house, Ho Chi Minh city, 152 pp. [in Vietnamese]

Huong D. T. T., Quyen T. N. T., 2012 [The effect of salinity on the embryonic development and osmoregulatory of the striped catfish (*Pangasianodon hypophthalmus*) larvae and fingerling stages]. Can Tho University Journal of Science 21b:29-37. [in Vietnamese]

Huong D. T. T, Ha N. T. K., Ngoc N. M., Em N. T., Kaneko T., Phuong N. T., 2020 [Effects of temperature on physiology, growth performance and digestive enzyme activity of striped catfish (*Pangasianodon hypophthalmus*) from fry to small fingerling stage]. Can Tho University Journal of Science, Aquaculture and Fisheries 56(2):1-11. [in Vietnamese]

Ibrahim A., Narayanan M., Salman K., Mohamed S., 2020 The effect of freshwater acidification in the tissues of *Cyprinus carpio*. Global Journal of Zoology 5(1):9-15.
Kennedy C. J., Picard C., 2012 Chronic low pH exposure affects the seawater readiness of juvenile Pacific sockeye salmon. Fish Physiology and Biochemistry 38:1131-1143.
Khang N., Ung P. D., Dung B. T. N., 1998 [Soil resources of Vietnam-Orient to land sustainable use]. Conference on integrated nutrition management to improve yield and quality of agricultural products in Vietnam - Challenges and opportunities. Nha Trang, Vietnam, June 16-18, 1998. [in Vietnamese]
Lam N. C., Huong D. T. T., Son V. N., Phuong N. T., 2011 [Physiological changes and growth of tra catfish (*Pangasianodon hypophthalmus*) exposed to different salinities]. Can Tho University Journal of Science 17a:60-69. [in Vietnamese]
Lefevre S., Jensen F. B., Huong D. T. T., Wang T., Phuong N. T., Bayley M., 2011 Effects of nitrite exposure on functional haemoglobin levels, bimodal respiration, and swimming performance in the facultative air-breathing fish *Pangasianodon hypophthalmus*. Aquatic Toxicology 104(1-2):86-93.
Lemieux H., Blier P., Dutil J. D., 1999 Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*)? Fish Physiology and Biochemistry 20(4):293-303.
Lopes J. M., Silva L. V. F., Baldisserotto B., 2001 Survival and growth of silver catfish larvae exposed to different water pH. Aquaculture International 9(1):73-80.
MARD, Ministry of Agriculture and Rural Development, 2017 Acid sulfate soil and salinity soil in The Mekong Delta. Available at: http://www.cuctrongtrot.gov.vn/TinTuc/Index/3611. Accessed: March, 2022.
Menendez R., 1976 Chronic effects of reduced pH on brook trout. Journal of the Fisheries Research Board of Canada 33:118-123.
Minh V. Q., Vu P. T., 2015 [Effective use of acid sulfate soil and salinity soil in the Mekong Delta]. National conference soils of Viet Nam: Current status and challenges. Nov, 2015. Agricultural publisher, pp. 167-174. [in Vietnamese]
Ndubuisi U. C., Chimezie A. J., Chinedu U. C., Chikwem I. C., Alexander U., 2015 Effect of pH on the growth performance and survival rate of *Clarias gariepinus* fry. International Journal of Research in Biosciences 4(3):14-20.
Norrgren L., Degerman E., 1992 The influence of liming on embryos and yolk sac fry of Atlantic salmon and brown trout in acidified river in Sweden. Journal of Fish Biology 23:567-576.
Nystrand M. I., Österholm P., 2013 Metal species in a Boreal river system affected by acid sulfate soils. Applied Geochemistry 31:133-141.
Parra J. E. G., Baldisserotto B., 2007 Effect of water pH and hardness on survival and growth of freshwater teleosts. In: Fish osmoregulation. Baldisserotto B., Mancera J. M., Kapoor B. G. (eds), New Hampshire: Science Publishers, pp. 135-150.
Phuc N. T. H., 2015 Effects of temperature and salinity on growth performance in cultured tra catfish (*Pangasianodon hypophthalmus*) in Vietnam. Doctoral dissertation, Queensland University of Technology, Brisbane, Australia, 131 pp.
Phuc N. T. H., Huong D. T. T., Mather P. B., Hurwood D. A., 2014 Experimental assessment of the effects of sublethal salinities on growth performance and stress in cultured tra catfish (*Pangasianodon hypophthalmus*). Fish Physiology and Biochemistry 40(6):1839-1848.
Phuong L. M., Huong D. T. T., Nyengaard J. R., Bayley M., 2017 Gill remodelling and growth rate of striped catfish *Pangasianodon hypophthalmus* under impacts of hypoxia and temperature. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 203:288-296.
Sahu S., Datta S., 2018 Effect of water pH on growth and survival of *Trichogaster lalius* (Hamilton, 1822) under captivity. International Journal of Current Microbiology and Applied Sciences 7:3655-3666.
Sammut J., Melville M. D., Callinan R. B., Fraser G. C., 1995 Estuarine acidification: impacts on aquatic biota of draining acid sulphate soils. Australian Geographical Studies 33(1):89-100.

Tseng H. C., Grendell J. H., Rothman S. S., 1982 Food, duodenal extracts, and enzyme secretion by the pancreas. American Journal of Physiology 243:304-312.
VASEP, Vietnam Association of Seafood Exporters and Producers, 2020 [Striped catfish culture situation]. Available at: https://vasep.com.vn/san-pham-xuat-khau/ca-tra/tong-quan-nganh-ca-tra. Accessed: March, 2022. [in Vietnamese]
White I., Melville M. D., Wilson B. P., Sammut J., 1997 Reducing acidic discharges from coastal wetlands in eastern Australia. Wetlands Ecology and Management 5:55-72.
Wood C. M., 2001 Toxic responses of the gill. In: Target organ toxicity in marine and freshwater teleosts. Volume 1 - Organs. Schlenk D., Benson W. H. (eds), Taylor & Francis, London, pp. 1-89.
Worthington T. M., 1982 Enzymes and related biochemicals. Biochemical Products Division, Worthington Diagnostic System, Freehold, NJ, USA, pp. 215-226.
Yen D. T., 2003 [Survey on some characters, morphology, growth and physiology of basa fish (*Pangasius bocourti*), striped catfish (*Pangasius hypophthamuls*) and their hybrids]. Master's thesis in Aquaculture. Can Tho University, Vietnam, 60 pp. [in Vietnamese]
Zweig R. D., Morton J. D., Stewart M. M., 1999 Source water quality for aquaculture. World Bank, Washington, 72 pp.

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Authors:
Nguyen Thi Kim Ha, Department of Applied Hydrobiology, College of Aquaculture and Fisheries, Can Tho University, Ninh Kieu district, Can Tho 900000, Vietnam, e-mail: kimha@ctu.edu.vn
Le Hong Thang, Department of Applied Hydrobiology, College of Aquaculture and Fisheries, Can Tho University, Ninh Kieu district, Can Tho city, Vietnam, e-mail: thangm0620008@gstudent.ctu.edu.vn
Nguyen Tinh Em, Department of Applied Hydrobiology, College of Aquaculture and Fisheries, Can Tho University, Ninh Kieu district, Can Tho city, Vietnam, e-mail: ntem@ctu.edu.vn
Tran Trung Giang, Department of Applied Hydrobiology, College of Aquaculture and Fisheries, Can Tho University, Ninh Kieu district, Can Tho city, Vietnam, e-mail: trunggiang@ctu.edu.vn
Nguyen Thanh Phuong, Department of Coastal Aquaculture, College of Aquaculture and Fisheries, Can Tho University, Ninh Kieu district, Can Tho city, Vietnam, e-mail: ntphuong@ctu.edu.vn
Do Thi Thanh Huong, Department of Applied Hydrobiology, College of Aquaculture and Fisheries, Can Tho University, Ninh Kieu district, Can Tho city, Vietnam, e-mail: dtthuong@ctu.edu.vn

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