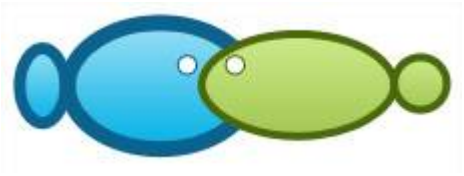

Effects of combined acidic sulfate water and salinity on the growth, survival and digestive enzyme activities of a salinity-tolerant striped catfish (*Pangasianodon hypophthalmus*) strain at the fingerling stage

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Abstract. A strain of salinity-tolerant striped catfish (*Pangasianodon hypophthalmus*) (fingerling size; 15.5±0.09 g each) was used for this experiment. The effects of combined acidic sulfate water and salinity on fish growth performance, survival rate, and digestive enzyme activities were assessed. Selected fish were allocated in a completely randomized design with triplicates of nine treatments, including three pH levels (5.5, 6.5 and 7.5) combined with three salinity levels (3, 6 and 9‰). The results indicate that there was an interaction effect of pH and salinity on the growth parameters, survival and feed conversion ratio (FCR) of fish after 60 days of culture ($p < 0.05$). Fish in the treatment with pH 6.5 and 3‰ salinity presented the highest weight (52.1±0.56 g), while the lowest weight was observed in the treatment with pH 5.5 and 9‰ salinity. Similarly, daily weight gain (DWG) and specific growth rate (SGR) were highest in the pH 6.5 and 3‰ salinity treatment ($p < 0.05$). There were significant differences among all treatments ($p < 0.05$) regarding FCR. The lowest and highest FCR values were in the treatments with pH 6.5 and 3‰ salinity (1.04) and pH 5.5 and 9‰ salinity (1.8), respectively. Fish in the treatment of pH 5.5 and 9‰ salinity showed very low survival rates when compared to other treatments. The results suggest that acidic sulfate water with a slightly low pH (pH 6.5) and salinity of 3‰ could be considered a suitable environment for salinity-tolerant striped catfish. Moreover, our results confirm that a slightly acidic sulfate water environment with a pH of 6.5 is good for catfish culture.

Key Words: amylase, chymotrypsin, growth performance, pH, *Pangasianodon hypophthalmus*.

Introduction. Striped catfish (*Pangasianodon hypophthalmus*) is an important commercial freshwater fish cultured in the Mekong Delta and has been considered one of the world's most successful aquaculture developments (De Silva & Phuong 2011). The meat products from this species have been exported to 140 countries (Hasan et al 2019). The export value of striped catfish in 2022 was US$ 1.54 million from a farming area of approximately 5400 ha (VASEP 2021). However, the striped catfish farming industry has been facing many serious challenges, including increasing salinity intrusion in the culture area due to climate change (Hai et al 2020) and water contamination from acidity (MARD 2017). According to RCP 8.5 (RCP - Representative Concentration Pathways), it has been predicted that an increase in temperature and sea level rise would mostly be concentrated in this delta due to the sea level rise projected to be 73 cm (49-103 cm) along the entire coastal area by 2100, resulting in salinity intrusion into inland areas (MONRE 2016). As a consequence, some aquaculture industries could be impacted, especially in freshwater aquaculture regions. Although Hieu et al (2021) revealed that larval striped catfish could be reared up from 5 to 10‰ salinity, a study by Ha et al (2021) showed an increasing trend in growth when striped catfish were exposed to salinities ranging from 0 to 9‰. Furthermore, Hai et al (2022) reported that salinity-tolerant striped catfish had a better tolerance to salinity when compared to freshwater striped catfish.

Notably, the area of acidic sulfate soils in Vietnam was found to be 1.8 million hectares, accounting for 5.5% of the country's total area. Over 1.6 million hectares of this type of land exist in the Mekong Delta region, with pH being as low as 3.5 (Khang et al 1998; MARD 2017). Drainage from these acidic sulfate soil regions lower water pH, which could interfere with its aquatic biota, especially after heavy rain events flush massive amounts of sulfuric acid and toxic, heavy metals into water associated with acid sulfate soil, resulting in fish mortality (White et al 1997), diseases (e.g., outbreaks of epizootic ulcerative syndrome), as well as reproduction, recruitment and growth problems for cultured species (Sammut et al 1995). The effects of lower pH have been reported in previous studies. For example, Sahu & Datta (2018) noted the optimal pH range of juvenile *Trichogaster lalius* to be 6.5 to 7.0, while Ndubuisi et al (2015) reported an optimal pH range of 6.0 to 7.0 for *Clarias gariepinus* fry. The growth performance of silver catfish (*Rhamdia quelen*) was significantly lower when the fish were raised at pH 6.0 and 7.0 when compared to higher pH levels, and deaths were found at pH 5.5 after 21 days of exposure (Lopes et al 2001). Copatti et al (2005) studied the influences of low pH on *R. quelen* and concluded that fish reared at pH 5.2-6.5 presented lower growth when compared to fish cultured at pH 7.5. Darmawan et al (2021) reported that a decrease in catfish (*Pangasius* sp.) survival was found when pH reached 4.0, with all experimental fish dying at a pH below 3.0. Additionally, reduced pH resulted in an increase in the plasma cortisol concentration due to the non-specific immunity of fish (Brown et al 1990).

However, these studies only focused on a single factor. As such, the present study aimed to elucidate the combined effects of acidic sulfate water and salinity at different levels on the growth and survival of a salinity-tolerant strain of striped catfish at the fingerling stage.

Material and Method

Experimental materials. The study was conducted from June 2022 to September 2022 at the College of Aquaculture and fisheries – Can Tho University. Selected salinity-tolerant striped catfish fingerlings from a selective breeding programme for salinity tolerance at Can Tho University were used in this study. Briefly, fish were reared in 10‰ salinity water until broodstock size (3-5 kg fish^{-1}). The broodstock were then cultured for maturation in 5‰ salinity water and spawning was artificially induced. After hatching, larvae were reared in a freshwater environment until the fingerling stage with a body weight of 15 to 20 g.

High-salinity water (80-100‰) was purchased from Vinh Chau district, Soc Trang province. Saline water was treated with chlorine (30 ppm) and aerated for 24 hours. The disinfected saline water was then diluted with tap water to the desired salinities.

Acidic sulfate soils were collected from an acid-contaminated location in Hoa An district, Hau Giang province. Acidic sulfate soils were mixed with tap water (ratio 1:1, v:v) to obtain highly acidic water with a pH of approximately 2.5-3.0. This low-pH water was then diluted with tap water to obtain various experimental pH levels.

Experimental design. The experiment was assigned in a completely randomised design with a triplicate of nine treatments. The experiment was conducted with three pH levels (5.5, 6.5 and 7.5) combined with three salinity (S) levels (3, 6 and 9‰). The experimental system was designed as a simple recirculating system consisting of three cultured tanks and one settling tank. All tanks had the same volume of 500 L (containing 300 L of water). Each tank was stocked with 40 fish. Salinity in the experimental tanks was increased by 1‰ every 8 hours, starting with higher-salinity treatments and followed by the lower-salinity treatments to have all the treatments reach the desired salinities simultaneously. Regarding pH levels, acidic sulfate water with low pH (2.5-3) was diluted with tap water to achieve the target pH levels. The pH in the lowest pH treatments was reduced first by 0.25 units every 2 hours until reaching the desired pH, followed by treatments with higher pH levels. The experimental period commenced when

the salinity and pH in all the treatments reached the desired levels. The experimental period was 60 days.

Fish were fed twice daily at 8:00 am and 4:00 pm at a saturated level using a commercial pellet (Aquaxcel-Cargill, coded 7444, 30% crude protein). After 30 minutes, uneaten pellets in the tanks were removed and quantified for the feed conversion ratio (FCR) calculation. Water was changed twice a week by siphoning 30% at the bottom and replacing it with the appropriate salinity and acidic sulfate water for each treatment.

Sample analysis methods

Water quality parameters. The water quality parameters (e.g., pH and temperature) were recorded daily using a LAQUAtwin pH-22 (Horiba, Japan) meter at 8.00 am and 2.00 pm. Dissolved oxygen (DO) was measured by WTW-conventional portable meters ProfiLine Oxi 3205 twice per week. Nitrite (NO_2^-) and total ammonia nitrogen (TAN) were measured once per week using the Griess llosvay, Diazonium and Indophenol blue methods, respectively. Sulfate (SO_4^{2-}) was measured using the method of APHA (2017), while total Al and Fe at the beginning and end of the experiment were measured using the methods described by APHA (1995).

Growth parameters. Growth sampling was accomplished on days 30 and 60 of the experimental period. Individual weight was recorded to the nearest gram. At the end of the experiment, the number of fish in each tank was counted to calculate the survival rate (SR). The growth performance was determined based on parameters such as daily weight gain (DWG), specific growth rate (SGR), SR and FCR:

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

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

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

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

where: W_0 is the initial weight (g), Wt is the final weight (g) and t is the experimental time (day).

Digestive enzymatic activities. Three fish were randomly collected from each tank at the end of the experiment. Feeding ceased for 24 hours before the sampling. The stomach and anterior part of the fish intestine were dissected to analyse the activity of pepsin and amylase in the stomach, as well as amylase, trypsin and chymotrypsin in the intestine. Samples were cleaned in distilled water and adipose tissues were cautiously removed. The samples were homogenated (IKA T10 basic Ultra-Turrax) 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°C. The supernatant was collected and stored at -80°C until the analysis of enzyme activities and protein content. Sample preparations were conducted at low temperatures on ice. Chymotrypsin and pepsin activities were measured by the method described by Worthington (1982), trypsin was measured using the method of Tseng et al (1982), amylase was measured using the method of Bernfeld (1951), and the protein content in the samples was analysed using the method of Bradford (1976). The specific activities of the enzymes are expressed as U mg protein^{-1} min^{-1}.

Statistical analysis. Microsoft Excel 2016 was used to conduct the descriptive statistical analysis, including standard deviation (SD) and mean (M). Two-way analysis of variance (ANOVA) together with Duncan's post-hoc tests were used to test for significant differences among treatments (at the 95% significance level) using SPSS 20.0.

Results

Water parameters. The pH levels were controlled according to the experimental design with a variation of ±0.01 during the experimental period. The temperature in the morning and afternoon fluctuated slightly, ranging from 27.94 to 28.00°C and 28.41 to 28.44°C, respectively. The average DO in culture tanks fluctuated from 6.29±0.12 to 6.59±0.12 mg L^{-1} (Table 1).

Table 1

Values of pH, temperature and DO in experimental tanks during the experiment

Treatment (pH-salinity)	*pH*		*Temperature (°C)*		*DO (mg L^{-1})*
	Morning	*Afternoon*	*Morning*	*Afternoon*	
5.5-3‰	5.55±0.01	5.57±0.01	27.95±0.01	28.41±0.01	6.44±0.17
6.5-3‰	6.56±0.01	6.54±0.01	27.95±0.01	28.42±0.00	6.29±0.12
7.5-3‰	7.49±0.01	7.51±0.01	27.97±0.01	28.42±0.00	6.42±0.23
5.5-6‰	5.56±0.01	5.57±0.01	27.97±0.05	28.43±0.03	6.39±0.20
6.5-6‰	6.54±0.01	6.56±0.01	28.00±0.05	28.44±0.03	6.59±0.12
7.5-6‰	7.48±0.01	7.50±0.01	27.94±0.06	28.43±0.03	6.44±0.26
5.5-9‰	5.54±0.01	5.55±0.01	28.00±0.06	28.44±0.04	6.45±0,29
6.5-9‰	6.53±0.01	6.54±0.01	28.00±0.06	28.44±0.03	6.40±0.23
7.5-9‰	7.49±0.01	7.51±0.01	27.98±0.01	28.43±0.01	6.55±0.07

Values are presented as mean±SD.

The nitrite concentration ranged from 0.05±0.00 to 0.18±0.01 mg L^{-1} during the study. TAN and ion sulfate (SO_4^{2-}) ranged from 0.27±0.04 to 0.71±0.02 mg L^{-1} and 157.13± 14.6 to 460.65±31.5 mg L^{-1}, respectively. The results indicate that the pH 5.5 treatments had higher concentrations of TAN and SO_4^{2-} due to requiring a large amount of acidic sulfate water to reach pH desired range. The concentrations of total Fe and Al^{3+} declined as the pH levels increased, with values of 0.17±0.01 to 0.27±0.03 mg L^{-1} and 0.61±0.03 to 1.45±0.09 mg L^{-1}, respectively (Table 2).

Table 2

Concentrations of TAN, NO_2^-, SO_4^{2-}, Al^{3+} and total Fe in experimental tanks during the experiment

Treatment (pH-salinity)	*TAN (mg L^{-1})*	*NO_2^- (mg L^{-1})*	*SO_4^{2-} (mg L^{-1})*	*Total Fe (mg L^{-1})*	*Al^{3+} (mg L^{-1})*
5.5-3‰	0.62±0.05	0.05±0.00	243.41±28.0	0.25±0.01	1.20±0.11
6.5-3‰	0.39±0.01	0.07±0.01	196.90±13.7	0.18±0.01	0.77±0.04
7.5-3‰	0.27±0.03	0.07±0.01	157.13±14.6	0.17±0.01	0.61±0.03
5.5-6‰	0.66±0.12	0.05±0.01	331.37±20.8	0.25±0.01	1.36±0.11
6.5-6‰	0.62±0.06	0.05±0.03	242.02±26.3	0.20±0.02	1.00±0.11
7.5-6‰	0.52±0.02	0.09±0.01	183.67±5.28	0.18±0.01	0.79±0.07
5.5-9‰	0.71±0.09	0.13±0.02	460.65±31.5	0.27±0.03	1.45±0.09
6.5-9‰	0.64±0.01	0.15±0.01	287.71±10.4	0.25±0.01	1.11±0.06
7.5-9‰	0.57±0.05	0.18±0.01	201.01±10.0	0.20±0.02	0.93±0.08

Values are presented as mean±SD.

Growth performance. The statistical results indicate that the pH and salinity levels significantly affected the weight of fish. Additionally, there was an interaction effect of these two factors on the investigated parameters ($p < 0.05$). After 30 days, the average individual fish weight increased from 15.5±0.09 to 29.9±0.74 g depending on the treatment. Mean fish weight in treatments 6.5-3‰ and 6.5-6‰ was higher than that of the other treatments (29.9±0.74 g and 29.6±1.40 g, respectively). The fish weights of these treatments were statistically significantly different from those of all other treatments ($p < 0.05$), except for the 5.5-3‰ treatment (28.0±1.80 g). The lowest mean weight was recorded in the 6.5-9‰ treatment (24.6±0.52 g) (Figure 1A).

After 60 days, fish weight ranged from 38.7±0.82 g to 52.1±0.54 g. The highest weight was observed in the 6.5-3‰ treatment (52.1±0.54 g), which is significantly

higher than those of the other treatments ($p < 0.05$). The lowest value was observed in the 5.5-9‰ treatment (38.7±0.82 g), which is significantly different from the other treatments ($p < 0.05$). Fish weights in the 6.5-6‰ and 7.5-3‰ treatments were 46.5±0.14 g and 43.9±0.70 g, respectively, with significant differences observed among all treatments ($p < 0.05$). The results demonstrate that acidic sulfate water with pH values lower than 6.5 negatively influenced the growth performance of salinity-tolerant striped catfish.

Feed conversion ratio. There was an interaction effect between acidic sulfate water and salinity levels on the FCR of fish ($p < 0.05$). The FCR was lowest (1.04±0.07) in the 6.5-3‰ treatment and highest (1.81±0.1) in the 5.5-9‰ treatment, which was significantly different from the other treatments ($p < 0.05$) (Figure 1B).

Daily weight gain and specific growth rate. An interaction between pH levels and salinity on the DWG of fish was observed on day 60 ($p < 0.05$). The 6.5-3‰ treatment showed the highest value (0.61±0.01 g day^{-1}) and was statistically different among all treatments ($p < 0.05$). The lowest value (0.38±0.01 g day^{-1}) was observed in the 5.5-9‰ treatment and was significantly lower than the other treatments ($p < 0.05$) (Figure 1C). Similarly, the interaction effect between acidic sulfate water and salinity on the SGR of fish was also observed on day 60 ($p < 0.05$). The SGR of fish reared in the 6.5-3‰ treatment showed the best performance (2.02±0.02 % day^{-1}), which was statistically different when compared to the other treatments ($p < 0.05$). The lowest SGR was recorded in the 5.5-9‰ treatment (1.52±0.03‰ day^{-1}) (Figure 1D). The results illustrate a declining trend in DWG and SGR from 3 to 9‰ salinity at different pH values (Figure 1C, D).

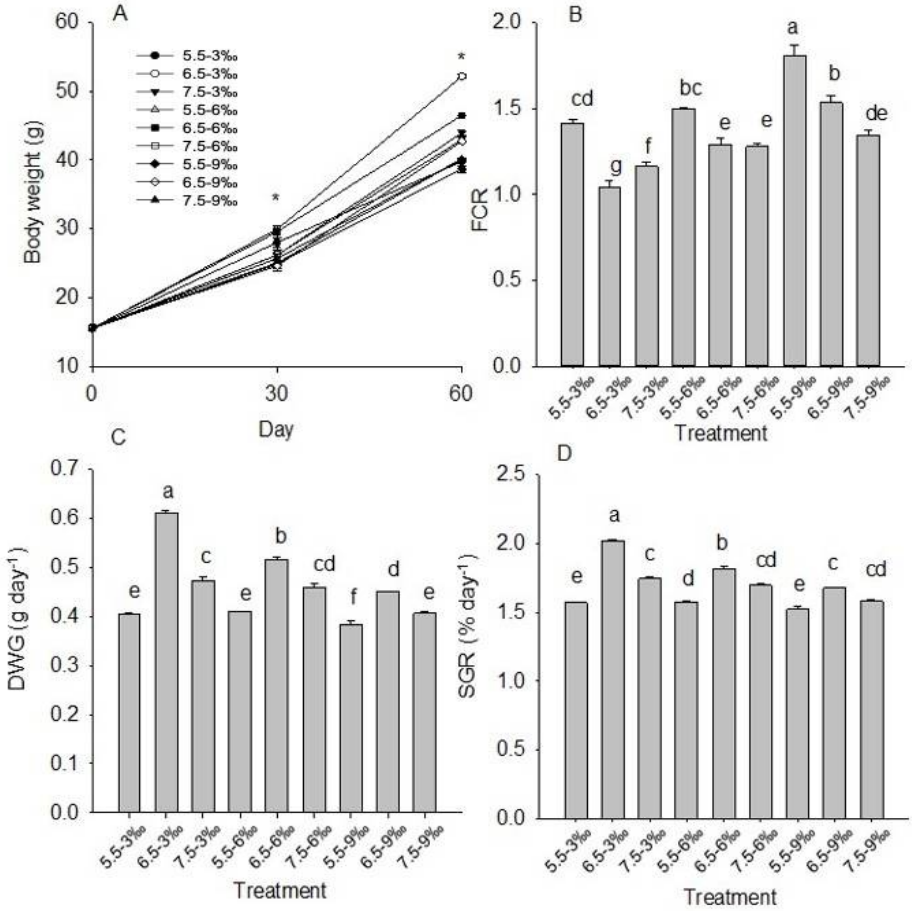

Figure 1. Average body weight (A), FCR (B), DWG (C) and SGR (D) of striped catfish cultured at different pH and salinity levels. The asterisk in the line and different letters (a, b, c, …) above the bars signify a significant difference ($p < 0.05$).

Survival rate. The SR of fish reared in the 5.5-9‰ treatment (80%) was significantly lower than those of the other treatments ($p < 0.05$). There were no significant differences in the SRs of the other treatments ($p > 0.05$).

Digestive enzyme activities. The results show no interaction between pH and salinity on enzymatic activities ($p > 0.05$) (Table 3). The lowest trypsin activity in the pH 5.5 treatment (0.0046±0.0012 U mg protein^{-1} min^{-1}) was significantly different from the other treatments ($p < 0.05$). The highest trypsin activity was highest in the 3‰ salinity treatment (0.0068±0.0019 U mg protein^{-1} min^{-1}) ($p < 0.05$). Moreover, the highest chymotrypsin activity was observed at pH 6.5 (312±90.8 U mg protein^{-1} min^{-1}), followed by pH 7.5 (263±37.3 U mg protein^{-1} min^{-1}) ($p < 0.05$). The 9‰ salinity treatment had the lowest chymotrypsin activity (234±38.3 U mg protein^{-1} min^{-1}) and was significantly different when compared to that in the 3‰ salinity treatment (317±71.9 U mg protein^{-1} min^{-1}) ($p < 0.05$). There was no significant influence of different pH and salinities on intestinal amylase activities of fish at the end of the experiment ($p > 0.05$). The amylase activity in the stomach was highest in the 3‰ salinity treatment (0.939±0.100 U mg protein^{-1} min^{-1}), followed by the 6‰ salinity treatment (0.914±0.050 U mg protein^{-1} min^{-1}) and was significantly different when compared to the 9‰ salinity treatment ($p < 0.05$). Regarding pH factor, the highest amylase activity in the stomach of fish was observed in the pH 6.5 treatment (0.943±0.080 U mg protein^{-1} min^{-1}) and was significantly different from the other treatments ($p < 0.05$). Concerning the individual effect, pepsin enzyme activity in the stomach of fish was not affected by pH but only by salinity. In particular, the pepsin activity decreased with an increase in salinity ($p < 0.05$). Pepsin activity in the 9‰ salinity treatments (0.11±0.008 U mg protein^{-1} min^{-1}) and 3‰ salinity treatments (0.14±0.006 U mg protein^{-1} min^{-1}) were the lowest and highest, respectively ($p < 0.05$).

Table 3

Digestive enzyme activities of salinity-tolerant striped catfish cultured at different pH and salinity levels

	Treatments (pH-salinity)	*Trypsin*	*Chymotrypsin*	*Amylase in intestine*	*Amylase in stomach*	*Pepsin*
pH × S	5.5-3‰	0.0046±0.0005	274±24.0	0.86±0.15	0.960±0.036	0.14±0.01
	6.5-3‰	0.0074±0.0010	395±71.0	0.92±0.28	1.003±0.035	0.14±0.02
	7.5-3‰	0.0086±0.0009	282±37.3	0.87±0.11	0.853±0.071	0.15±0.01
	5.5-6‰	0.0043±0.0022	261±28.4	0.87±0.08	0.877±0.050	0.14±0.02
	6.5-6‰	0.0061±0.0013	298±82.7	1.19±0.20	0.973±0.095	0.14±0.01
	7.5-6‰	0.0063±0.0016	261±51.6	1.08±0.11	0.893±0.045	0.12±0.02
	5.5-9‰	0.0049±0.0010	212±31.2	0.91±0.07	0.753±0.031	0.11±0.01
	6.5-9‰	0.0061±0.0004	243±57.5	0.96±0.16	0.853±0.055	0.12±0.02
	7.5-9‰	0.0055±0.0001	246±22.5	0.92±0.24	0.870±0.095	0.11±0.01
pH	5.5	0.0046±0.0012^{a}	249±37.2^{a}	0.88±0.09	0.863±0.100^{a}	0.13±0.02
	6.5	0.0065±0.0011^{b}	312±90.8^{b}	1.02±0.23	0.943±0.080^{b}	0.13±0.01
	7.5	0.0065±0.0016^{b}	263±37.3^{ab}	0.96±0.17	0.872±0.050^{a}	0.13±0.02
S	3‰	0.0068±0.0019^{B}	317±71.9^{B}	0.88±0.17	0.939±0.100^{B}	0.14±0.006^{C}
	6‰	0.0053±0.0015^{A}	273±54.1^{AB}	1.05±0.18	0.914±0.050^{B}	0.13±0.011^{B}
	9‰	0.0055±0.0007^{A}	234±38.3^{A}	0.93±0.15	0.826±0.070^{A}	0.11±0.008^{A}
P-value	pH	0.002	0.036	0.233	0.025	0.386
	S	0.039	0.008	0.129	0.003	0.000
	pH × S	0.214	0.317	0.640	0.064	0.240

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

Discussion

Water parameters. The water temperature and DO values in this study fluctuated within the normal range for fish. Huong et al (2020a) reported that the appropriate temperature range for striped catfish from fry to small fingerlings is between 27 and

32°C. Mallya (2007) recommended that oxygen levels for tropical freshwater species be above 5 mg L^{-1}. Moreover, Boyd (1998) suggested that nitrite concentrations should not exceed 0.30 mg L^{-1} in aquaculture ponds. As suggested by Boyd (1998), SO_4^{2-} concentrations in all treatments were higher than the appropriate concentration for aquatic organisms, which is 5-100 mg L^{-1}. Some previous studies reported that heavy metals such as aluminium and iron were toxic to aquatic animals when they exceed a certain limit (Smith et al 1973; Gostomski 1990; Botté et al 2022). The maximum total iron concentration should not exceed 1.0 mg L^{-1} to protect aquatic systems from the detrimental effects of iron (EPA 1986). On the other hand, Smith et al (1973) observed that more than 1.0 mg L^{-1} iron concentrations affected the feeding of fry and juveniles, causing prolonged stress and reduced growth. Van Dam et al (2018) recommended a water quality guideline value for dissolved aluminium in seawater of 56 µg L^{-1} to protect 95% of marine species. Gostomski (1990) reported that the average concentration of aluminium does not exceed 0.75 mg L^{-1} when the ambient pH is between 6.5 and 9.0 to protect from acute toxicity. Botté et al (2022) stated that aluminium becomes more soluble and toxic when pH decreases. Generally, these water quality parameters during the experiment are within acceptable limits for aquatic species. However, the aluminium concentration in all treatments (except for the 6.5-3‰ treatment) was over the acceptable limit (0.75 mg L^{-1}), which could have had a negative effect on the fish.

Growth performance. Salinity and pH levels are considered crucial water quality parameters that serve significant roles in the physiology and growth of fish. The current study highlights the strong effects of the interactions of salinity and pH levels on the growth performance of striped catfish, with better performance shown in the condition of 3‰ salinity and pH 6.5. This finding is supported by the results of previous research. For example, Phuc (2015) demonstrated that striped catfish in the control (13.5±5.36 g) and 2‰ salinity (11.6±3.38 g) treatments exhibited the best weight gain when compared to other treatments with higher salinity treatments. According to Jahan et al (2019), the SGR of striped catfish (14.9±1.36 g) was significantly higher in 4‰ salinity (0.81± 0.04%), followed by 0, 8 and 12‰ salinity ($p < 0.05$). Moreover, Ha et al (2021) showed that larval striped catfish reared at salinities of 0 and 3‰ had the best weight gain when compared to higher salinity treatments. Notably, the salinity-tolerant striped catfish strains used in this study proved to have a high salinity tolerance, which was highlighted by the ability of these fish to survive and grow rapidly in saline water. Hai et al (2022) showed that fry to fingerling stage salinity-tolerant striped catfish from a selected group (broodstocks had the best growth from the selection programme at 10‰ salinity) and a random group (broodstocks were randomly selected from the selection programme in the condition of 10‰ salinity) had SR, growth rate, and FCR values that were better than those in the freshwater group (broodstocks and fry were completely reared in freshwater) ($p < 0.05$) when fish were reared in 0, 5, 10, 15 and 20‰ salinity. Regarding pH, the highest growth indicators in this study were observed in the pH 6.5 treatment when compared to the pH 5.5 and 7.5 treatments. The results of this study are similar to the report of Ha et al (2022), which was the first study to investigate the effect of acidic sulfate water (low pH) on the growth performance of striped catfish. That study showed that striped catfish presented significantly higher growth in terms of weight and length in acidic sulfate water in the pH 6.5 treatment ($p < 0.05$), with the lowest growth being shown in the pH 4.5 treatment. Furthermore, an investigation by Lopes et al (2001) noted that silver catfish larvae (*R. queen*) exposed to pH 5.5 reduced in growth parameters when compared to those raised in pH 6.0 and 7.0 water. In this study, the weight gain of salinity-tolerant striped catfish in treatment 6.5-3‰ was 36.6 g, which was higher than that of normal striped catfish fingerlings (freshwater striped catfish; 31.5 g) reared at the same salinity level (3‰) by Phuong et al (2023), pH level (6.5; using acidic sulfate water; 29 g) by Ha et al (2022). This proves the high tolerance of salinity-tolerant striped catfish against the investigated factors (salinity and acidic sulfate water) when compared to the normal striped catfish.

Survival rate and feed conversion ratio. The SR was lowest in the 5.5-9‰ treatment when compared to the other treatments. Craig & Baksi (1977) stated that flagfish (*Jordanella floridae*) fry survival declined ($p < 0.05$) at pH 5.5 and 5.0, and no fry survived at pH 4.5. According to Darmawan et al (2021), striped catfish exposed to gradual pH decreases illustrated mortality within 240 minutes in the pH 3.0 and 4.0 treatments, which had significantly higher mortality than the other treatments ($p < 0.05$). Moreover, low salinities (2-10‰) were considered optimal conditions for the survival of striped catfish, while the 0 and 14‰ salinity treatments had poorer SRs (Phuc et al 2014). These results may indicate that an individual factor of 9‰ salinity or pH 5.5 did not affect the survival of striped catfish. However, a pH of 5.5 combined with 9‰ salinity adversely affects the survival of salinity-tolerant striped catfish when compared to treatments with higher pH and lower salinity. During the first period of the experiment, fish had to use a lot of energy to acclimate to their new environments, which led to the mortality of some fish. Moreover, the 5.5-9‰ treatment had the highest aluminium concentration when compared to the other treatments. Teien et al (2005) showed that high salinity levels combined with low pH and high aluminium concentration caused high aluminium accumulation in fish gills, respiratory failure and high fish mortality, which explains the decreased survival rates of fish in the 5.5-9‰ treatment in this study.

Regarding FCR, the results indicated that combining a salinity level of 3‰ and a pH of 6.5 resulted in the lowest FCR. Ha et al (2022) reported that the FCR of striped catfish fingerlings reared in acidic sulfate water was highest (1.64) at pH 4.5, while the lowest FCR (1.08) was observed at pH 6.5. There was an increasing trend in FCR between pH and salinity levels among all treatments. Sahu & Datta (2018) reported that *Trichogaster lalius* fingerlings had the lowest FCR (1.02) in the pH 7 treatment and the highest FCR (4.8) at pH 5, with these differences being significant from the pH 6, 8 and 9 treatments ($p < 0.05$). Ahirwal et al (2021) found that FCR had an increasing trend from 0 to 6‰ salinity, with values ranging from 2.18±0.15 to 3.16±0.11 in the freshwater fish *Gibelion catla*. Moreover, Phuc et al (2014) found that salinities from 0 to 10‰ did not affect FCR in striped catfish; however, FCR decreased when fish were exposed to higher salinities (14 and 18‰).

Digestive enzymatic activities. Acidic sulfate water with low pH and salinity had a combined effect on growth, survival, and FCR in salinity-tolerant striped catfish; however, digestive enzyme activity was only affected by individual salinity or pH factors. In this study, the intestinal amylase activities were not significantly influenced by neither salinities nor pH levels ($p > 0.05$). These results are similar with the findings of Ha et al (2022) that amylase enzyme activities in the intestine of striped catfish were not impacted by different pH levels from 4.5 to 7.21. On the other hand, the activities of the digestive enzymes in the intestine (trypsin and chymotrypsin) and stomach (amylase and pepsin) decreased in the high salinity (9‰) treatment if compared to 3 and 6‰ salinity treatments. The current results are similar to the research of Hoa (2014) on striped catfish fingerlings (8-10 g). After 70 days of rearing, amylase enzyme activity, chymotrypsin, and pepsin in the 12 and 15‰ salinity treatments tended to decrease and were different from the treatments with lower salinity. Moreover, in a study of cultured snakehead fish (*Channa striata*) fry to fingerlings, Huong et al (2020b) showed that amylase enzyme activity, trypsin and chymotrypsin tended to decrease with high salinity after 90 days of culture. The intestinal trypsin enzyme activity was highest in the 0‰ salinity treatment and decreased significantly when the salinity increased to the 6 and 9‰ levels. According to Ha et al (2021), the striped catfish fry showed decreased chymotrypsin and trypsin under high salinity ($p < 0.05$), with the lowest values exhibited under 15‰ salinity. Previous studies have shown that fish spend 10-50% of their energy budget on homeostatic activity for adapting to an increased salinity environment. Fish drank more water and increased ion excretion through the expenditure of energy and matter for the Na^+/K^+-ATPase channel (Bœuf & Payan 2001). Some studies have noted that fish living in environments with increasing salinity will need to purposefully drink water to compensate for water loss. The drinking rates of fish increased after they were transferred from freshwater to saline water, which increased further after some time with

saline water contact (Evans 1975, 1984, 1993; Payan et al 1984). This alters the pH in the fish digestive system, which affects digestive enzyme activity (Bath & Eddy 1979; Bœuuf & Payan 2001). Thus, the results of this study are consistent with the aforementioned studies since salinity affected the digestive enzyme activity of salinity-tolerant striped catfish. Trypsin, chymotrypsin and amylase (in the stomach) activity of striped catfish decreased when salinity increased to 9‰. Changes in habitat salinity can affect many physiological processes and cause stress in fish (Bœuuf & Payan 2001). In addition to the salinity factor, an acidic sulfate water environment (through a low pH factor) also affects the digestive enzyme activity of fish. In the present experiment, the salinity-tolerant striped catfish culture at pH 6.5 had the highest digestive enzyme activity when compared to those reared at pH 5.5 and 7.5. Another study of striped catfish fingerlings showed an increase in trypsin and chymotrypsin activity with pH increasing from 4.5 to 6.5, with trypsin and chymotrypsin activity being highest at pH 6.5 when compared to pH 4.5 and 5.0 (Ha et al 2022). The pH values in the stomachs and intestines of all fish species are in the range of 3.5-4.5 and 6.5-7.2, respectively, while the optimum pH for stomach enzyme activity in many fish species ranges from 2 to 4 (Castillo-Yañez et al 2004). In addition, pH also affects the secretion of digestive enzymes through changes in the structure of the intestine. Studies on carp (*Cyprinus carpio*) showed that there was a change in intestine morphology when fish were cultured at different pH values (6.6, 5.8 and 5.0). Research has shown the occurrence of necrosis and damage to the intestinal mucosa when fish are cultured at low pH values. This effect leads to a decrease in the concentration of enzymes secreted and thus interferes with the digestive process (Ibrahim et al 2020). Abbink et al (2012) reported that low pH affected growth performance, appetite and food conversion efficiency, and disruption to physiological homeostasis in fish. In this study, these factors reduced growth and increased the FCR of salinity-tolerant striped catfish.

Conclusions. The combination of salinity and acidic sulfate water affects the growth performance, survival rate and FCR of salinity-tolerant striped catfish. The digestive enzyme activity of fish decreased gradually in high salinity and low pH conditions. The treatment of 3‰ salinity and pH 6.5 is likely the optimal condition for salinity-tolerant striped catfish. Our results confirm that a slightly acidic sulfate water environment with pH 6.5 is a good condition for striped catfish culture.

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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. C., 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 330-333:130-135.

Ahirwal S. K., Das P. C., Sarma K., Kumar T., Singh J., Kamble S. P., 2021 Effect of salinity changes on growth, survival and biochemical parameters of freshwater fish Gibelion catla (Hamilton, 1822). Journal of Environmental Biology 42(6):1519-1525.

APHA, 1995 Standard methods for the examination of water and wastewater. 19th edition. American Public Health Association, Washington DC, 1000 pp.

APHA, 2017 Standard methods for the examination of water and wastewater. 23th edition. American Public Health Association, Washington DC, 1504 pp.

Bath R. N., Eddy F. B., 1979 Salt and water balance in rainbow trout (*Salmo gairdneri*) rapidly transferred from fresh water to sea water. Journal of Experimental Biology 83(1):193-202.

Bernfeld P., 1951 Enzymes of starch degradation and synthesis. Advances in Enzymology and Related Areas of Molecular Biology 12:379-428.
Bœuf G., Payan P., 2001 How should salinity influence fish growth? Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 130(4):411-423.
Botté A., Zaidi M., Guery J., Fichet D., Leignel V., 2022 Aluminium in aquatic environments: abundance and ecotoxicological impacts. Aquatic Ecology 56(3): 751-773.
Boyd C. E., 1998 Water quality for pond aquaculture. Research and Development Series, No. 43. International Center for Aquaculture and Aquatic Environments, Alabama Agricultural Experiment Station, Auburn University, 37 pp.
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.
Brown S. B., MacLatchy D. L., 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(7):1537-1543.
Castillo-Yañez F. J., Pacheco-Aguilar R., Garcia-Carreño F. L., de los Angeles Navarrete-Del M., 2004 Characterization of acidic proteolytic enzymes from Monterey sardine (*Sardinops sagax caerulea*) viscera. Food Chemistry 85(3):343-350.
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.
Craig G. R., Baksi W. F., 1977 The effects of depressed pH on flagfish reproduction, growth and survival. Water Research 11(8):621-626.
Darmawan J., Pribadi T. D. K., 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.
De Silva S. S., Phuong N. T., 2011 Striped catfish farming in the Mekong Delta, Vietnam: a tumultuous path to a global success. Reviews in Aquaculture 3(2):45-73.
Evans D. H., 1975 Ionic exchange mechanisms in fish gills. Comparative Biochemistry and Physiology Part A: Physiology 51(3):491-495.
Evans D. H., 1984 The roles of gill permeability and transport mechanisms in euryhalinity. In: Fish physiology. Volume 10, Part B. Hoar W. S., Randall D. J. (eds), Academic Press, New York, pp. 239-283.
Evans D. H., 1993 Osmotic and ionic regulation. In: The physiology of fishes. Evans D. H. (ed), CRC Press, Boca Raton, FL, pp. 315-341.
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., 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 13:1-9.
Ha N. T. K., Thang L. H., Em N. T., Giang T. T., Phuong N. T, Huong D. T. T., 2022 Effects of acidic sulfate water on growth, survival, and digestive enzyme activities of striped catfish (*Pangasianodon hypophthalmus*) fingerlings. AACL Bioflux 15(2): 819-829.
Hai D. M., Yen D. T, Liem P. T., Tam B. M., Son V. N., Huong D. T. T., Hang B. T. B., Tran N. T. N., Hieu D. Q., Kestemont P., Phuong T. N., Farnir F., 2022 Selective breeding of saline-tolerant striped catfish (*Pangasianodon hypophthalmus*) for sustainable catfish farming in climate vulnerable Mekong Delta, Vietnam. Aquaculture Reports 25:101263.
Hai T. N., Phuong N. T., Hoa N. V., Viet L. Q., Khanh L. V., Tao C. T., Anh N. T. N., Thao N. T. T., Sorgeloos P., 2020 Promoting Coastal Aquaculture for Adaptation to Climate Change and Saltwater Intrusion in the Mekong Delta, Vietnam. World Aquaculture 51:19-26.

Hasan M. R., Shipton T. A., Bueno P. B., 2019 Aquafeed value chain analysis and a review of regulatory framework of striped catfish farming in Viet Nam. FAO Fisheries and Aquaculture Technical Paper, No. 648, 58 pp.
Hieu D. Q., Hang B. T. B., Huong D. T. T., Kertaoui N. E., Farnir F., Phuong N. T., Kestemont P., 2021 Salinity affects growth performance, physiology, immune responses and temperature resistance in striped catfish (*Pangasianodon hypophthalmus*) during its early life stages. Fish Physiology and Biochemistry 47(6):1995-2013.
Hoa H. T. T., 2014 [Effect of salinity on digestive enzyme activities of striped catfish (*Pangasianodon hypophthalmus*)]. Master thesis of Aquaculture, Can Tho University, 63 pp. [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]
Huong D. T. T., Ky T. M., Ha N. T. K., Em N. T., Yasuaki T., Phuong N. T., 2020b [Effects of salinity on physiological parameters, digestive enzyme activities and growth of snakehead fish (*Channa striata*)]. Can Tho University Journal of Science 56(1):11-19. [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.
Jahan A., Nipa T. T., Islam S. M. M., Uddin M. H., Islam M. S., Shahjahan M., 2019 Striped catfish (*Pangasianodon hypophthalmus*) could be suitable for coastal aquaculture. Journal of Applied Ichthyology 35(4):994-1003.
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]
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.
Mallya Y. J., 2007 The effects of dissolved oxygen on fish growth in aquaculture. The United Nations University, Fisheries Training Programme, Final Project, 30 pp.
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.
Payan P., Girard J. P., Mayer-Gostan N., 1984 Branchial ion movements in teleosts: the roles of respiratory and chloride cells. In: Fish physiology. Volume 10, Part B. Hoar W. S., Randall D. J. (eds), Academic Press, New York, pp. 39-63.
Phuc N. T. H., 2015 Effects of temperature and salinity on growth performance in cultured tra catfish (*Pangasianodon hypophthalmus*) in Vietnam. Doctoral thesis, 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 N. T., Ha N. T. K., Huong D. T. T., 2023 Growth and feed intake of striped catfish (*Pangasianodon hypophthalmus*) fingerlings reared in different salinities. AACL Bioflux 16(1):496-507.
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.

Smith E. J., Sykora J. L., Shapiro M. A., 1973 Effect of lime neutralized iron hydroxide suspensions on survival, growth, and reproduction of the fathead minnow (*Pimephales promelas*). Journal of the Fisheries Research Board of Canada 30(8): 1147-1153.
Teien H. C., Salbu B., Heier L. S., Kroglund F., Rosseland B. O., 2005 Fish mortality during sea salt episodes - catchment liming as a countermeasure. Journal of Environmental Monitoring 7(10):989-998.
Tseng H. C., Grendell J. H., Rothman S. S., 1982 Food, duodenal extracts, and enzyme secretion by the pancreas. American Journal of Physiology 243(4):304-312.
van Dam J. W., Trenfield M. A., Streten C., Harford A. J., Parry D., van Dam R. A., 2018 Water quality guideline values for aluminium, gallium and molybdenum in marine environments. Environmental Science and Pollution Research 25(26):26592-26602.
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.
Worthington T. M., 1982 Enzymes and related biochemicals. Biochemical Products Division, Worthington Diagnostic System, Freehold, NJ, USA, pp. 215-226.
*** EPA (Environmental Protection Agency, USA), 1986 Quality criteria for water. Office of Water Regulations Standards Washington, DC 20460. Water, EPA 440/5-84-001.
*** 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). [in Vietnamese]
*** MONRE (Ministry of Natural Resources and Environment, Hanoi, Vietnam), 2016 [Climate change, sea level rise scenarios for Vietnam]. NARENCA Publisher, 188 pp. [in Vietnamese]
*** VASEP (Vietnam Association of Seafood Exporters and Producers), 2022 [An overview of *Pangasius* industry]. Available at: https://vasep.com.vn/san-pham-xuat-khau/ca-tra/tong-quan-nganh-ca-tra. Accessed: April, 2022. [in Vietnamese]

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