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Hatching success and growth of Snakehead (*Channa lucius* Cuvier, 1831) larvae and fry at different pH levels

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Abstract – Snakehead (*Channa lucius* Cuvier, 1831) is a species of potential aquaculture interest in the Mekong Delta, Vietnam. However, their optimum environmental conditions have yet to be determined. This study aims to study the hatching success and larval and fry growth of *C. lucius* at six pH levels (5.5, 6.0, 6.5, 7.0, 7.5, and 8.0). Two consecutive experiments were conducted. Experiment 1 focused on incubating eggs and nursing the yolk sac larvae, and was carried out in aquariums. The monitoring included incubation time (IT), hatching rate (HR), and deformation rate of the newly hatched larvae (DR). Larvae were also collected daily during nursing for growth determination. In Experiment 2, four-day-old fry after hatching from experiment 1 were reared in plastic tanks with live feeds for 30 days. Growth was checked every ten days, while survival rate (SR) and coefficient of variation (CV) were determined at the end of the experiment. pH 5.5–8.0 was favorable for incubation, larvae, and fry, as assessed through good indicators of IT, HR, DR, and larval length and weight growths, as well as length and weight growths, SR, and CV of fry. Furthermore, the lowest DR (0.33%) was at pH 5.5, while except for pH 7.0, the shortest IT (41.1 h) significantly differed from that at higher pH levels ($p < 0.05$), and the highest larval growth parameters were at pH 5.5–6.0; meanwhile, pH 5.5–6.5 supported a better life for fry, as showed by the significantly higher growth parameters ($p < 0.05$) and SR and CV improvements in fry after 30 rearing days. Overall, pH 5.5–8.0 was suitable for the early stages of *C. lucius*, in which pH 5.5–6.0 was better for incubation and larval growth and pH 5.5–6.5 was better for fry development.

Keywords: *Channa lucius* / newly hatched / deformity / coefficient of variation / Vietnam

1 Introduction

The Snakehead (*Channa lucius* Cuvier, 1831), together with three other fish species of the *Chana* genus (*C. striata*, *C. micropeltes*, and *C. gachua*) is a native species in Vietnam (Tran et al., 2013). This fish group is a very popular food source because their flesh contains high nutritional content, low fat content, few intramuscular spines, and even very high levels of albumin, a compound that is widely applied in medicine for a role in wound healing (Zuraini et al., 2006; Agustin et al., 2016; Purwanti et al., 2019). *C. lucius* is known to be tolerant of drastically low oxygen environments. Because it possesses a highly vascularized air-breathing organ for high gas exchange (Mohsin and Ambak, 1983), an ideal trait for high-density intensive farming. Natural populations of *C. lucius* are widepread throughout Southern Asia, southern China, Indochina, and the Sunda Islands (Mohsin and Ambak, 1983). They often occupy ponds, lakes, peat swamps, well-shaded forest streams, and middle zones of rivers, where temperatures are between 24 and 29 °C and pH is between 5.6 and 6.0 or less (Lee and Ng, 1994), with slow water flow, abundant aquatic plants, and rotting wood (Rainboth, 1996).

In recent years, the natural stocks of *C. lucius* in Vietnam have been severely reduced because of overfishing and decreasing habitat due to human activities and other factors due to climate change (Ngoc-Tran and Thuy-Yen, 2016). This fish species is currently only often encountered populating and breeding wildly in inland water supplying canals where there is little water exchange, a low pH (around 5.4–5.7), dissolved

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oxygen of 3.4–5.50 mg L^{-1}, and a temperature of 29.3–30.3 °C (Ly, 2016). Also, Ly (2016) only achieved success in the artificial propagation trials of *C. lucius* in mediums with a pH range of 5.5–6.0 without success at higher pH levels in the Mekong Delta, Vietnam. So, in addition to the above-mentioned reasons for the decrease of natural stocks, specific pH conditions for proper development and reproduction is probably one of the reasons why natural populations have become very limited, leading *C. lucius* to be currently classified as endangered in Vietnam (Vietnam MARD, 2011).

Freshwater aquaculture has been considered a traditional production sector in Vietnam and one of the country's key export commodity productions (Anh et al., 2016; Tri et al., 2021). In 2019, Vietnam was the third-largest seafood exporter in the world by value, with a total output of fisheries reaching 8.27 million MT, of which freshwater aquaculture accounted for 54.31% (4.49 million MT) and cultured fish accounted for 69.86% (3.14 million MT) (GSO, 2021). The Mekong Delta region in southern Vietnam is known as a wetland ecosystem with an abundance of fresh water, making it ideal for the development of freshwater fish (Sinh et al., 2014; Anh et al., 2016; Tri et al., 2021). While Tra catfish (*Pangasius hypophthalmus*) is a primary economic freshwater fish for export (Tan et al., 2020), Snakehead has received high attention from domestic markets. Two Snakehead species, *C. striata* and *C. micropeltes,* have been domesticated and bred successfully, and they have also been commonly farmed in different rearing systems, such as earthen ponds, hapas, cages, tanks, and rice fields (Ly, 2016; Tan et al., 2020). However, *C. lucius* is still known as a wild fish.

Besides useful freshwater resources, the Mekong Delta still has a rather large area (approximately 1.6 million ha) of highly acid sulfate soils (Sumida et al., 2012) that cover more than 40% of the region's agricultural land, acidifying the receiving waters (Guong and Hoa, 2012; Trinh, 2017; Thao et al., 2020). Even in several small rivers and canals in some of the Mekong Delta provinces, the pH levels of the surface water are commonly only 4.5–5.5 (Trinh, 2017), leading to limitations in aquaculture and agriculture production in the area.

pH is one of the key parameters of water quality that require strict monitoring in aquaculture production because it impacts cultured animals both directly and indirectly (Fondriest Environmental Inc, 2013). It is suggested that the ideal pH range for most aquatic animals, including fish, is near neutral (Das et al., 2006; Boyd, 2013), and acute or chronic exposure to acidic water can adversely affect their physiological functions and reduce hatching, growth, and survival (Fondriest Environmental Inc, 2013; Kim et al., 2020; Prakoso et al., 2020). Meanwhile, some authors pointed out that the impacts of pH on animals usually differ between species, vary depending on a variety of specific ecological factors in the areas where they populate (Beltrão et al., 2019), and can relate to their natural history (Reynalte-Tataje et al., 2015). It was estimated that over 1000 acid-tolerant fish species from over 40 divergent families survive and thrive in the Rio Negro of the Amazon River system (Beltrão et al., 2019), where pH is around 4.5–5.1 (Kwong et al., 2014). Kwong et al. (2014) said that thanks to their specialized physiological mechanisms in ionic regulation and acid-base balance in habitats, the fish can thrive in acidic water in the Rio Negro. This knowledge showed the great potential of *C. lucius*, a fish species often inhabiting freshwaters with low pH levels (around 5.5–6.0) (Lee and Ng, 1994; Ly, 2016), to become an important candidate species for aquaculture in acidic waters in the Mekong Delta. However, little work has been done to date on domesticating this species for aquaculture production. Previous studies focused on aspects such as live feeding (Azrita and Syandri, 2013) and stimulating spawning using LHRHa (Azrita et al., 2014). In Vietnam, Ly (2016) performed a research series on the basic aspects of *C. lucius*, such as natural habitat, nutrition spectrum, gastrointestinal development, artificial reproduction with stimulating hormones, and the possibility of using artificial animal feed. Aquaculture diversification is one of the promising strategies in Vietnam. Furthermore, naturally acidic freshwaters are found worldwide (Nelson, 2015). The aim of our study was to investigate the optimum pH conditions for optimal hatching and growth performance of larvae and fry in *C. lucius*. It is an important step for the development of commercial aquaculture of this fish species in the Vietnamese Mekong Delta and similar regions.

2 Materials and methods

2.1 Experimental materials

The fish eggs used in Experiment 1 came from semi-artificially spawned broodstock at the experimental hatchery of the Faculty of Fisheries at Can Tho University, Vietnam.

10 L glass aquariums were used for incubation and nursing the larvae from newly hatched to yolk sac exhaustion, and the 60 L plastic tanks were used for rearing from fry to juvenile.

The source of water used for the experiments was tap water with a pH level of 8, which was continuously strongly aerated for 24 h to remove residual chlorine, followed by adding H_3BO_4 at a dose of 720 mL per m^3 to create water with a pH level of 5.5. Then water with a pH level of 5.5 was mixed with the initial chlorine-removed tap water (pH level of 8) to obtain water meeting other experimental pH levels of 6.0, 6.5, 7.0, and 7.5 using the following formula: $C_1V_1 = C_2V_2$ (C: concentration, V: volume).

The US National Research Council's guide for the Care and Use of Laboratory Animals was followed.

2.2 Experimental design

The lowest pH value chosen in our study was based on the pH ranges where *C. lucius* commonly encounters wild reproduction or successful artificial reproduction in the Mekong Delta, Vietnam, around 5.5–6.0 (Ly, 2016). We investigated the effects of six different pH levels (5.5, 6.0, 6.5, 7.0, 7.5, and 8.0) at two early life stages of *C. lucius*. Two experiments with three randomized replicates for each condition were consecutively performed from April to June 2014 at the experimental hatchery of the Faculty of Fisheries at Can Tho University, Vietnam, as follows.

Experiment 1: effects of pH on incubation time (IT), hatching rate (HR), and deformation rate of newly hatched larvae (DR), and larval growth from newly hatched to yolk sac exhaustion

Experiment 1 was conducted in eighteen 10 L glass aquariums with eight liters of water in late April 2014. The inseminated eggs of *C. lucius* obtained from the semi-artificial reproduction were washed with clean water and arranged at a density of 150 eggs per aquarium. During the experiment period, the aquariums were continuously lightly aerated, and the water was changed every six hours, with 30% of the water volume in the aquariums. After ten hours of incubation, the damaged eggs with milky whites were regularly removed by siphoning. After hatching, at each pH level, ten newly hatched larvae from each aquarium were randomly collected to be observed for eventual deformations under the microscope at a magnification of 100x. In addition, the newly hatched larvae continued to be held for care in the aquariums until their yolk sacs were empty to determine their growth. During the care period, twenty larvae were collected daily to determine growth parameters. The larval weight was weighed using an electrical analytical balance calibrated to 0.01 mg. The larval length was measured using biometric measurements under a microscope at a magnification of 10x with a millimeter unit. The monitored parameters in this experiment included IT, HR, DR, and the growth performance of the larvae from newly hatched to yolk sac exhaustion.

Experiment 2: effects of pH on growth and survival in the fry-to-juvenile stage

This experiment was conducted from early May to early June 2014. **The hatchlings after the four hatching days from Experiment 1** were arranged in eighteen 60 L plastic tanks, **each with 50 liters of water**, at a density of 2 individual L^{-1}. The fish were fed twice a day at 7:00 and 16:00 with live feeds to apparent satiation, including Moina for fish at the ages of 1–7 days and Tubifex for fish at the ages of 8–30 days, which were suitable feeds for the mouth size and nutritional needs at each stage of the fish (Ly, 2016). All tanks were continuously aerated and changed every three days with 20–30% of the tank volume. The rearing period was 30 days in order to limit changes in feed requirements and the cannibalism of the Snakehead fish in the larger stage in captivity (Raizada et al., 2022), which may affect the results. After every ten rearing days, ten fish from each plastic tank (30 fish of each pH level) were weighed using an electrical analytical balance calibrated to 0.01 mg and measured on a divided ruler of a millimeter unit to calculate their growth performance. The data for the calculations on SR and CV were collected at the end of the experiment.

2.3 Monitoring of water quality

In both experiments, water quality was checked prior to fish stocking and during the culture period. Temperature and pH were measured with an ECO pH meter, and dissolved oxygen was measured by a HANNA meter-98172, twice a day at 8:00 and 16:00. Besides, water samples were collected once a week at 8:00 for nitrite determination using the Griess-Llosvay method, spectrophotometrically at 530 nm.

2.4 Data calculations

The monitored data from two experiments was calculated using the following equations:

- IT was determined from the eggs being fertilized until the point of hatching at 5% (Kamler, 2002).
- HR (%) = (number of newly hatched larvae/number of stocked eggs at the beginning of incubation) $\times$100.
- DR (%) = (number of deformity larvae/total number of larvae observed) x 100.
- Weight gain = final weight − initial weight.
- Length gain = final length − initial length.
- Daily weight gain (g day^{-1}) = (final weight − initial weight)/experiment days.
- Daily length gain (cm day^{-1}) = (final length − initial length)/experiment days.
- SGR (% day^{-1}) = 100 $\times$ (ln (final weight or length) − ln (initial weight or length))/duration of experiment.
- SR (%) = (final fish number/initial fish number) $\times$100.
- CV (%) = (the standard deviation/the mean weight) $\times$ 100.

2.5 Statistical Analysis

The variance homogeneity was assessed by Levene's test, and the percentage data were transformed into arcsines prior to conducting statistics. The mean values at different pH levels were compared using a one-way analysis of variance (ANOVA), and the Duncan test ($p < 05$) indicated a significant difference using SPSS version 20.0.

3 Results

3.1 Water quality parameters

Over the experimental period, the monitored water quality variables in both experiments showed little changes. They included temperatures ranging from 28.51 to 28.54 °C in the morning and ranging from 28.9 to 29.2 °C in the afternoon, with the diurnal temperature fluctuation of less that 2 °C. Meanwhile, dissolved oxygen levels were always higher than 5 mg L^{-1} and ranged from 6.20 to 6.30 mg L^{-1} in the morning and 6.20 to 6.23 mg L^{-1} in the afternoon. Also, nitrite checked every week at 8:00 showed a change in the range from 0.30 to 0.38 mg L^{-1} (Tab. 1).

3.2 Effects of pH on eggs and larvae

Variations of incubation time (IT), hatching rate (HR), and deformation rate of newly hatched larvae (DR).

Data in Table 2 shows that IT, HR, and DR had values of 41.1–41.4 h, 89.6–92.9%, and $\leq$1%, respectively. pH levels clearly affected the IT and DR. The IT and DR increased with increasing pH levels. Specifically, the shortest IT in the low pH range of 5.5–6.0 (41.1 h) significantly differed from that in the higher pH range of 6.5–8.0 (41.2–41.4 h) ($p < 0.05$), except at pH 7.0 (41.1 h) ($p < 0.05$). DR was less than or equal to 1%, with the lowest value of 0.33% at pH 5.5, followed by 0.67% at pH levels 6.0, 6.5, and 7.5, and 1% at pH 7.0 and 8.0. Still, HR was less affected by the pH levels, which varied in a range of 89.6–92.9%, with the highest value (92.9%) at pH 7.0 and the lowest value (89.6%) at pH 8.0, and there was no significant difference among them.

3.2.1 Growth in length and weight of larvae

The larval duration from newly hatched to yolk sac exhaustion was defined as four days. Data presented in Table 3 shows that pH's effect on larval mean length increased toward rearing time as pH levels increased. Specifically, the mean

Table 1. Water quality variables during the rearing time of larvae and fry of *C. lucius* at different pH levels (mean ± SD).

pH	Temperature (°C)		Dissolved oxygen (mg L^{-1})		Nitrit (mg L^{-1})
	7:00	14:00	7:00	14:00	
5.5	28.5 ± 0.11	28.9 ± 0.12	6.20 ± 0.0	6.20 ± 0.05	0,30 ± 0,01
6.0	28.5 ± 0.00	29.1 ± 0.06	6.21 ± 0.4	6.21 ± 0.09	0,37 ± 0,04
6.5	28.5 ± 0.00	29.2 ± 0.00	6.23 ± 0.9	6.23 ± 0.04	0,37 ± 0,04
7.0	28.5 ± 0.00	29.1 ± 0.06	6.30 ± 0.9	6.21 ± 0.03	0,38 ± 0,05
7.5	28.5 ± 0.00	29.2 ± 0.01	6.30 ± 0.1	6.20 ± 0.04	0,37 ± 0,04
8.0	28.5 ± 0.03	29.2 ± 0.06	6.30 ± 0.3	6.20 ± 0.04	0,37 ± 0,04

Table 2. Incubation time (IT), hatching rate (HR), and deformation rate (DR) of *C. lucius* newly hatched larvae at different pH levels.

pH levels	IT (hours)	HR (%)	DR (%)
5.5	41.1 ± 0.00[c]	92.0 ± 3.79[a]	0.33
6.0	41.1 ± 0.06[c]	92.4 ± 5.19[a]	0.67
6.5	41.2 ± 0.06[b]	91.7 ± 6.11[a]	0.67
7.0	41.1 ± 0.06[c]	92.9 ± 3.48[a]	1.00
7.5	41.3 ± 0.10[ab]	91.2 ± 7.09[a]	0.67
8.0	41.4 ± 0.10[a]	89.6 ± 2.47[a]	1.00

Values are presented as mean ± SD. Means with different superscripts (a, b, c) in the same columns are statistically significant differences ($p < 0.05$).

length can be divided into two groups (at pH < 7.0 and pH≥7.0) on rearing day 1, followed by three groups (at pH<7.0, pH = 7.0, and pH ≥ 7.5) on rearing day 2, and four groups (at pH ≤ 6.0, pH = 6.5, pH = 7.0, and pH ≥ 7.5) on rearing days 3 and 4, respectively. Remarkably, the mean length at pH levels of 5.5 and 6.0 was always the highest and significantly differed from the higher pH levels during the stage, with values of 7.32 ± 0.01 and 7.33 ± 0.01 mm, respectively, on the last day of the stage. In contrast, the lowest values of 7.09 ± 0.02 mm were recorded at pH levels of 7.5 and 8.0 on the same day.

In the same pattern as for mean length, mean weight was also affected by high pH levels in the investigated range, in which pH ≥ 7.0 always strongly and significantly affected mean weight on three of the first rearing days ($p < 0.05$). However, on the final day of this stage, mean weight was significantly affected by pH ≥ 6.5 ($p < 0.05$) and reached the highest values at two pH levels of 5.5 and 6.0 (4,10 ± 0,01 mg) ($p > 0.05$), followed by two pH levels of 6.5 and 7.0 (4.08 ± 0.01 mg), and the lowest at the other two pH levels of 7.5 and 8.0 (4.05 ± 0.01 mg) ($p > 0.05$) (Tab. 3).

After four hatching days, the parameters of length gain, daily length gain, and SGR in length of larvae reached values of 2.32–2.10 mm, 0.52–0.58 mm day^{-1}, and 8.76–9.53% day−1, respectively, and showed to be better in the acidic to neutral pH range (5.5–7.0) and significantly higher in the acidic pH range (5.5–6.5) than those in the alkaline pH range (7.5–8.0) ($p < 0.05$) (Tab. 4).

The parameters of weight gain, daily weight gain, and SGR in weight of larvae after four hatching days were presented in Table 4, with value ranges of 1.76–1.81 mg, 0.440–0.457 mg day^{-1}, and 14.23–14.60% day^{-1}, respectively. These parameters always reached their highest values in the pH range of 5.5–6.0 and significantly decreased in order in the pH ranges of 6.5–7.0 and 7.5–8.0.

3.3 Effects of pH on the fry-to-juvenile stage

3.3.1 Growth performance

Recorded growth performance in length and weight of fry are presented in Figures 1, 2, and Tables 5, 6, showing that the effect of pH on the fish weight was similar to their length in the range of pH levels investigated. It meant that growth slowed as pH levels increased.

3.3.2 Growth performance in length

In the first ten rearing days, the fish achieved the longest mean length at pH levels 5.5 and 6.0 (1.07 and 1.06 cm, respectively), followed by pH levels 6.5 and 7.0 (0.97 cm), with an insignificant difference between them ($p > 0.05$); the shortest mean length was obtained at pH levels 7.5 and 8.0 (0.93 cm), which differed significantly from the length at pH 5.5 and 6.0 ($p < 0.05$) (Fig. 1). A similar variation pattern in fish mean length was observed in the following ten rearing days of culture. However, there was no significant difference in the fish mean length at pH levels, except at pH 5.5, where the fish mean length of 1.57 cm) was significantly longer than that at pH 8 (1.36 cm) ($p > 0.05$). After 30 rearing days, the longest fish mean length was recorded at pH levels 5.5 and 6.0, with the same value being 2.45 cm; and in the pH range of 6.5–8.0, fish mean length decreased gradually as pH levels rose,

Table 3. Mean length and weight of *C. lucius* larvae during the rearing period from newly hatched to yolk sac exhaustion at different pH levels.

pH levels	Newly hatched	Day 1	Day 2	Day 3	Day 4
Mean length (mm)					
5.5	5.00 ± 0.04^{a}	5.41 ± 0.03^{a}	6.11 ± 0.05^{ab}	6.80 ± 0.02^{a}	7.32 ± 0.01^{a}
6.0	5.02 ± 0.04^{a}	5.45 ± 0.05^{a}	6.15 ± 0.01^{a}	6.81 ± 0.03^{ab}	7.33 ± 0.01^{a}
6.5	5.03 ± 0.04^{a}	5.42 ± 0.02^{a}	6.11 ± 0.01^{ab}	6.76 ± 0.04^{b}	7.26 ± 0.01^{b}
7.0	4.97 ± 0.04^{a}	5.34 ± 0.04^{b}	6.08 ± 0.04^{b}	6.71 ± 0.03^{c}	7.17 ± 0.06^{c}
7.5	4.97 ± 0.04^{a}	5.32 ± 0.03^{b}	5.97 ± 0.02^{c}	6.61 ± 0.01^{d}	7.09 ± 0.02^{d}
8.0	4.97 ± 0.04^{a}	5.30 ± 0.03^{b}	5.97 ± 0.01^{c}	6.60 ± 0.02^{d}	7.09 ± 0.02^{d}
Mean weight (mg)					
5.5	2.28 ± 0.05^{a}	2.40 ± 0.01^{b}	2.62 ± 0.01^{a}	2.91 ± 0.01^{ab}	4.10 ± 0.01^{a}
6.0	2.29 ± 0.05^{a}	2.42 ± 0.01^{a}	2.62 ± 0.01^{a}	2.92 ± 0.01^{a}	4.11 ± 0.01^{a}
6.5	2.29 ± 0.05^{a}	2.40 ± 0.01^{b}	2.62 ± 0.01^{a}	2.90 ± 0.01^{b}	4.08 ± 0.01^{b}
7.0	2.28 ± 0.05^{a}	2.38 ± 0.01^{c}	2.59 ± 0.01^{b}	2.89 ± 0.02^{c}	4.08 ± 0.01^{b}
7.5	2.29 ± 0.05^{a}	2.38 ± 0.01^{c}	2.57 ± 0.02^{c}	2.86 ± 0.03^{d}	4.05 ± 0.01^{c}
8.0	2.29 ± 0.05^{a}	2.38 ± 0.01^{c}	2.57 ± 0.03^{c}	2.87 ± 0.04^{d}	4.05 ± 0.01^{c}

Values are presented as mean ± SD. Means with different superscripts (a, b, c, d) in the same columns are statistically significant differences ($p < 0.05$).

Table 4. Gain and growth rate of *C. lucius* larvae after four nursing days at different pH levels.

pH levels	Length gain (mm)	Daily length gain (mm day^{-1})	SGR in length (% day^{-1})
5.5	2.32 ± 0.06^{c}	0.58 ± 0.02^{c}	9.53 ± 0.30^{c}
6.0	2.30 ± 0.01^{bc}	0.58 ± 0.01^{bc}	9.41 ± 0.05^{bc}
6.5	2.24 ± 0.01^{bc}	0.56 ± 0.00^{bc}	9.22 ± 0.05^{abc}
7.0	2.19 ± 0.11^{ab}	0.55 ± 0.03^{ab}	9.12 ± 0.48^{abc}
7.5	2.10 ± 0.06^{a}	0.52 ± 0.02^{a}	8.76 ± 0.30^{a}
8.0	2.13 ± 0.04^{a}	0.53 ± 0.01^{a}	8.81 ± 0.19^{a}
	Weight gain (mg)	Daily weight gain (mg day^{-1})	SGR in weight (% day^{-1})
5.5	1.81 ± 0.00^{c}	0.450 ± 0.000^{b}	14.56 ± 0.00^{c}
6.0	1.82 ± 0.01^{c}	0.457 ± 0.006^{c}	14.60 ± 0.03^{c}
6.5	1.79 ± 0.01^{b}	0.450 ± 0.000^{b}	14.46 ± 0.03^{b}
7.0	1.79 ± 0.01^{b}	0.450 ± 0.000^{b}	14.42 ± 0.03^{b}
7.5	1.76 ± 0.00^{a}	0.440 ± 0.000^{a}	14.25 ± 0.00^{a}
8.0	1.76 ± 0.01^{a}	0.440 ± 0.000^{a}	14.23 ± 0.03^{a}

Values are presented as mean ± SD. Means with different superscripts (a, b, c, d) in the same columns are statistically significant differences ($p < 0.05$).

reaching the lowest value at pH 8 (1.90 cm), significantly different from that at pH 5.5–6.0 ($p < 0.05$) (Fig. 1).

The parameters of length gain, daily length gain, and SGR in length are presented in Table 5. The data shows a decreasing tendency for these parameters as the pH level increases. The length gain was associated with daily length gain and a SGR in length and resulted in a similar gain pattern during the rearing time of 30 days, which was divided into three groups. Specifically, the group involving the largest values was in the pH range of 5.5 to 6.5, the smaller values were in the pH range of 7.0 to 7.5, and the smallest was at pH 8.0. The difference between these three groups was statistically significant.

3.3.3 *Growth performance in weight*

Data in Figure 2 shows that after 10 and 20 days of rearing, the mean weights were quite small at all investigated pH levels, ranging from 9.20 to 14.40 mg and 28.70 to 39.20 mg, respectively. However, the mean weight rapidly rose in the final 30 days of rearing and ranged from 84.60 to 162.70 mg at the final sampling time.

During the period of small fish (after the ten rearing days), the weight of fish at pH levels 5.5 and 6.0 was equal (14.40 mg), higher than those at other pH levels, and significantly different from those at pH levels 7.0–8.0 (9.20–9.80 mg). In the 20 rearing days, the mean weight

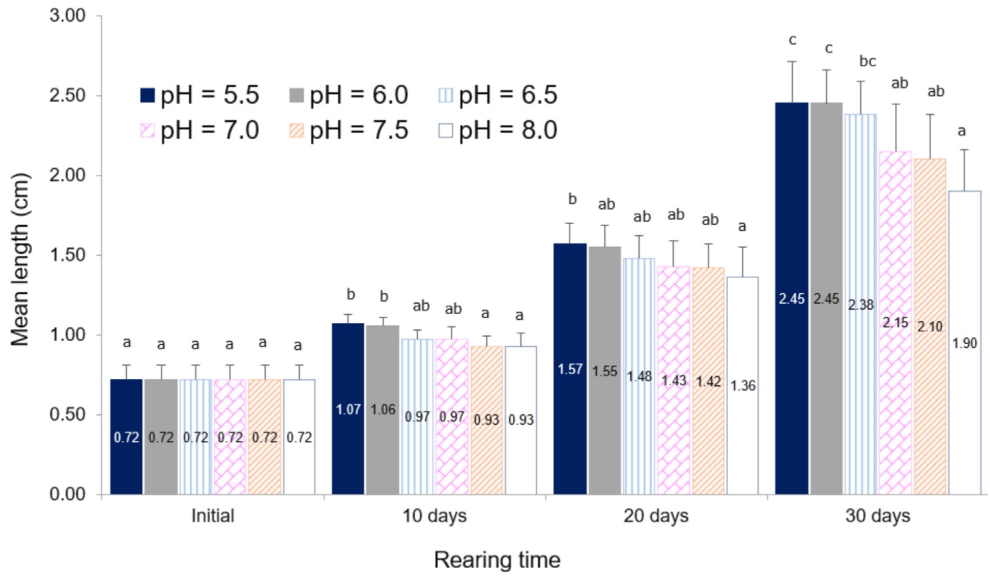

Fig. 1. Mean length of *C. lucius* fry during 30 rearing days at different pH levels. Values are presented as mean ± SD. Different letters (a, b, c) above bars at the same sampling time indicate significant differences ($p < 0.05$).

Table 5. Length gain, daily length gain, and SGR in length of *C. lucius* fry after 30 rearing days at different pH levels.

pH levels	Length gain (cm)	Daily length gain (cm day^{-1})	SGR in length (% day^{-1})
5.5	1.73 ± 0.03[a]	0.057 ± 0.001[a]	4.09 ± 0.05[a]
6.0	1.72 ± 0.08[a]	0.057 ± 0.002[a]	4.08 ± 0.11[a]
6.5	1.66 ± 0.01[a]	0.055 ± 0.000[a]	3.98 ± 0.11[a]
7.0	1.43 ± 0.03[b]	0.048 ± 0.006[b]	3.64 ± 0.31[b]
7.5	1.38 ± 0.03[b]	0.046 ± 0.001[b]	3.57 ± 0.15[b]
8.0	1.18 ± 0.03[c]	0.039 ± 0.001[c]	3.23 ± 0.05[c]

Values are presented as mean ± SD. Means with different superscripts (a, b, c) in the same columns are statistically significant differences ($p < 0.05$).

ranged from 28.70 mg to 37.50 mg and decreased as pH increased, but there were no significant differences among pH levels. On the last rearing day (after the 30 rearing days), a varied pattern in the mean weight at investigated pH levels was the same as those in the first ten rearing days. The values of 162.70 mg and 149.10 mg at pH levels 5.5 and 6.0, respectively, were higher than those at other pH levels and insignificantly different from those at pH level 6.5 (128.80 mg), but a significant difference compared to those at pH levels 7.0, 7.5, and 8.0 (with respective values of 108.90 mg, 98.70 mg, and 84.60 mg) (Fig. 2).

The parameters of weight gain, daily weight gain, and SGR in weight reached their highest values at pH 5.5, with values of 160.04 mg, 5.31 mg day^{-1}, and 12.3% day^{-1}, respectively. There was no significant difference compared to pH 6.0 (with respective values of 149.77 mg, 4.81 mg day^{-1}, and 12.0% day^{-1}), but a significant difference compared to pH 6.5–8.0. Moreover, at pH 8.0, weight gain (80.22 mg), daily weight gain (2.73 mg day^{-1}), and SGR in weight (10.1% day^{-1}) are equal to 50%, 50.1%, and 82.1% of those, respectively, at pH 5.5 (Tab. 6).

3.3.4 Survival rate (SR) and Coeffcient of variation (CV)

C. lucius fry reached rather high SRs at the pH range of 5.5–8.0, with ranges of 99.0–99.7%, 81.0–96.7%, and 69.0–90.7% after ten, twenty, and thirty rearing days, respectively (Fig. 3). There was no significant difference in SR among the investigated pH levels after the initial ten rearing days ($p > 0.05$). After 20 days of rearing, the SR reached the highest value at pH 6.5 (96.7%), insignificantly different from that at other pH levels except for pH 8 ($p < 0.05$) at 81.0%. At the end of the rearing period, the highest SR was obtained at pH 6.5 (90.7%), insignificantly different from that at lower pH levels 5.5 and 6.0 (with values of 88.3% and 81.7%, respectively), but significantly different from that at higher pH levels 7, 7.5, and 8, with respective values of 71.3%, 74.7%, and 69% (Fig. 3).

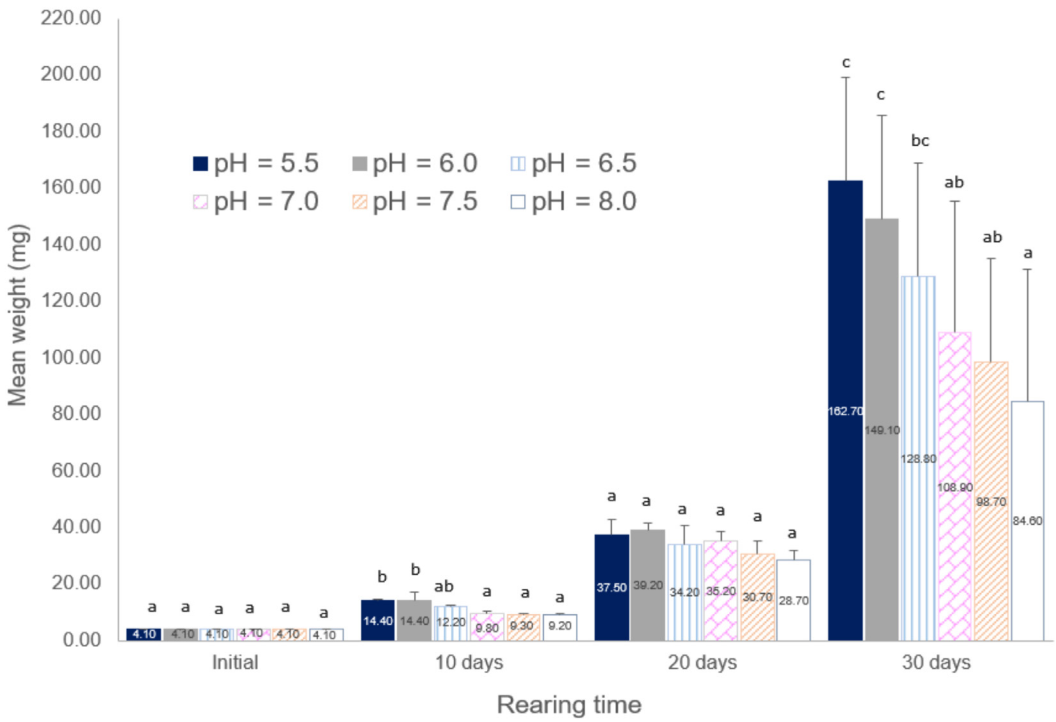

Fig. 2. Mean weight of *C. lucius* fry during 30 rearing days at different pH levels. Values are presented as mean ± SD. Different letters (a, b, c) above bars at the same sampling time indicate significant differences ($p < 0.05$).

Table 6. Weight gain, daily weight gain, and SGR in weight of *C. lucius* fry after 30 rearing days at different pH levels.

pH levels	Weight gain (mg)	Daily weight gain (mg day^{-1})	SGR in weight (% day^{-1})
5.5	160.04 ± 10^{a}	5.31 ± 0.14^{a}	12.3 ± 0.35^{a}
6.0	149.77 ± 11^{ab}	4.81 ± 0.11^{ab}	12.0 ± 0.37^{ab}
6.5	120.13 ± 10^{bc}	4.40 ± 0.12^{bc}	11.5 ± 0.05^{bc}
7.0	99.76 ± 12^{cd}	3.50 ± 0.12^{cd}	10.9 ± 0.76^{cd}
7.5	90.03 ± 15.57^{d}	3.23 ± 0.13^{de}	10.6 ± 0.42^{de}
8.0	80.22 ± 15.66^{d}	2.73 ± 0.11^{e}	10.1 ± 0.23^{e}

Values are presented as mean ± SD. Means with different superscripts (a, b, c, d, e) in the same columns are the statistically significant difference ($p < 0.05$).

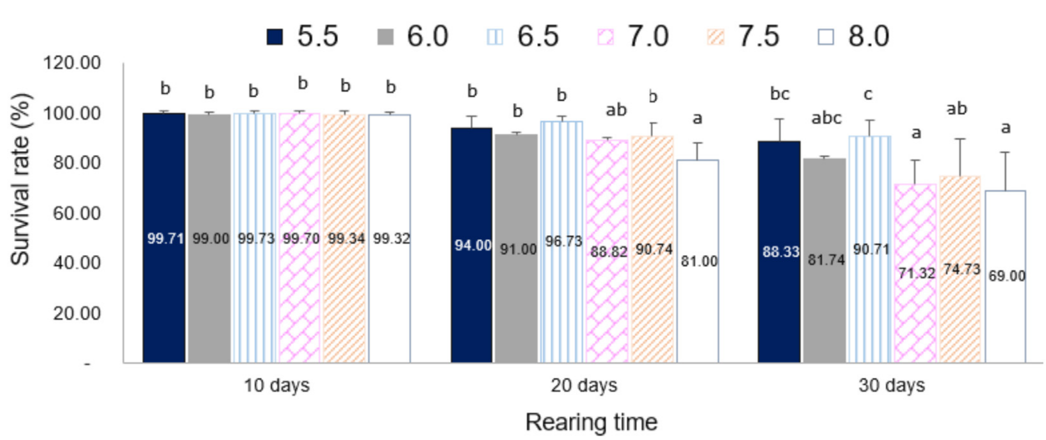

Fig. 3. Survival rate of *C. lucius* fry during 30 rearing days at different pH levels. Values are presented as mean ± SD. Different letters (a, b, c) above bars at the same sampling time indicate significant differences ($p < 0.05$).

Table 7. Coefficient of variation (CV) of *C. lucius* fry after 30 rearing days at different pH levels.

pH levels	5.5	6.0	6.5	7.0	7.5	8.0
CV (%)	23.3[a]	23.7[a]	28.0[a]	44.5[b]	36.9[ab]	54.9[bc]

Values are presented as mean ± SD. Means with different superscripts (a, b, c) in the same row are statistically significant differences ($p < 0.05$).

The CV was recorded at the end of the rearing and is presented in Table 7. It showed that CV fluctuated from 23.3% to 54.9%. Accordingly, with low (acidic) pH levels of 5.5, 6.0, and 6.5, CV was documented at values of 23.3%, 23.75, and 28.0%, respectively, insignificantly different among them ($p >$ 0.05). While at the higher (neutral and alkaline) pH levels of 7.0, 7.5, and 8.0, the recorded CV was at respective values of 44.5%, 36.9%, and 54.9%, there were no significant differences between them ($p > 0.05$), but they were significantly greater compared to those at the acidic pH levels, except at pH 7.5 ($p < 0.05$).

4 Discussion

4.1 Water quality parameters

Water is the lifeline of aquatic animals, including fish, and has direct effects on their lives. Water quality parameters measured in both experiments included temperature (28.5–29.2 °C), DO (>5 mg L^{-1}), and nitrite (0.3–0.38 mg L^{-1}) (Tab. 1). These values were to be favorable for the hatching of eggs and rearing of larvae and fry of Snakehead (Lee and Ng, 1994; Ly, 2016; Rahman et al., 2012; Hieu et al., 2022), and therefore they had little effect on the results of the experiments.

4.2 Effects of pH on the stages of eggs and larvae

4.2.1 Variations of incubation time (IT), hatching rate (HR), and deformation rate of newly hatched larvae (DR)

Table 2 showed that IT, HR, and DR all performed well in the pH range investigated. These implied that the pH range of 5.5–8.0 was favorable for embryonic development and newly hatched larvae of *C. lucius*. The HR (89.6–92.9%) in *C. lucius* larvae was within the HR ranges found in *C. striata* (50.76–93.18%, Rawat et al., 2020) and *C. punctatus* (84.3–96.3%, Marimuthu and Haniffa, 2010), while being higher than those in *C. bleheri* (65–85%, Nayak et al., 2020) and *C. striatus* (60.26%, Amornsakun et al., 2011). Also, IT (41.1–41.4 h) in C. *lucius* was longer than those in *C. striata* (17–26, Rawat et al., 2020; 20.12–30.01 h, Muslim, 2018) and *C. striatus* (28.4 h, Amornsakun et al., 2011), but shorter as compared to *C. argus* (45 h, Whedbee, 2017) and *C. bleheri* Vierke, 1991 (44–46 h, Nayak et al., 2020). Besides, DR (0.33–1%) in *C. lucius* was lower than those in many other fish species found by many previous authors (Kupren et al., 2010; Wu et al., 2019; Imsland et al., 2019; Kang et al., 2020).

The above-mentioned information suggests clear differences in IT and HR in fish species of the Channa genus. Furthermore, abiotic factors such as water temperature (Muslim et al., 2018), aerated conditions (Purnamawati et al., 2017), etc... are known to strongly affect the incubation and hatching performance of fish eggs. At lower pH levels (4.17–5.21) and a similar temperature range (28–30 °C) to our experiments, *C. striata* showed great performance in incubation, with an IT of 23.13–28.02 h, an HR of 82.00–86.33%, and 100% of newly hatched larvae without deformation (Muslim et al., 2018). It showed good acid resistance ability in Snakeheads in their early stages. In our results, the shortest IT (41.1 h) was observed at pH levels 5.5–6.0, which significantly differed from those at higher pH levels except at pH 7.0; simultaneously, the lowest DR (0.33%) was at pH 5.5 (Tab. 2). The observations in IT and DR indicated that low pH levels (5.5–6.0) were better for the embryonic development of *C. lucius* in our tested conditions.

4.2.2 Growth in length and weight of larvae

Data in SGR in length and weight (Tab. 4) showed *C. lucius* larvae grew rather well at pH levels from 5.5 to 8.0. Furthermore, larval growth showed to be higher in the pH range of 5.5–6.0 as compared to the higher pH range (6.5–8.0), as assessed through the variations in growth performance parameters at divergent pH levels after four nursing days. The larvae reached the highest mean length and weight, weight gain, daily weight gain, and SGR in weight at the pH range of 5.5–6.0, significantly decreased at the pH range of 6.5–7.0, and continued to significantly reduce to the lowest values at the pH range of 7.5–8.0 (Tabs. 3 and 4).

Thus, the findings in Experiment 1 proved that the pH range of 5.5–6.0 was better for developing embryos and larvae of *C. lucius* as compared to the higher pH levels (6.5–8.0). This can probably be related to the natural habitat of the species. It is suggested that each species has an ideal environmental range for growth related to its natural conditions (Reynalte-Tataje et al., 2015). Although numerous aquaculture researchers have said that the ideal pH range for most fish species is near neutral (Das et al., 2006; Boyd, 2013), in fact, an abundance of fish species thrive in the Rio Negro of the Amazon River system, where pH is below 5 (Kwong et al., 2014; Beltrão et al., 2019). Several authors have highlighted that ionic regulation and acid-base balance are fundamental to the physiology of the acclimation of fish to acidic waters (Kwong et al., 2014; Duarte et al., 2018; Morris et al., 2021). Through a systematic overview of zebrafish *Danio rerio*, a representative of acid-resistant fish species in the Rio Negro, Kwong et al. (2014) summarized that thanks to a complex network of specialized regulatory responses, including regulation of transcellular and paracellular Na^+ movement, modulation of gene expression, intracellular remodeling, cell proliferation, and hormonal regulation, these responses ultimately enabled Zebrafish to maintain Na^+ balance during acclimation to acidic environments. Besides their ability to acclimate to acidic water via physiological mechanisms, the abundance of acid-resistant species, like most other aquatic animals, is often confined to specific ecological environments. Relevant decisive factors include water body types (main rivers, tributary rivers, lakes, swamps, etc.), historical and geomorphological factors, hydrological periods, water chemistry, etc. in the waters (Beltrão et al., 2019). It may be the case with *C. lucius*, a fish species often encountered inhabiting and reproducing naturally in waters with a pH range of 5.5–6.0 or less (Lee and Ng, 1994; Ly, 2016), and it only achieved successful artificial reproduction in a medium with a similar pH range without success at pH higher levels (Ly, 2016). Therefore, the early stages of their life cycles (embryos and larvae) were observed to have better performance at pH 5.5–6.0 as compared to higher pH levels in the present work.

4.3 Effects of pH on the fry-to-juvenile stage

4.3.1 Growth performance

At the end of the rearing period, the SGR in weight (10.1–12.3% day^{-1}) was higher in *C. lucius* fry as compared to *C. punctatus* (Rahman et al., 2018; Marimuthu and Haniffa, 2006), *C. striatus* (Muntaziana et al., 2013), and *C. striata* (Helkianson et al., 2020). In addition, growth parameters (mean length and weight, length gain, daily length gain, and SGR in length) at pH 7.0–8.8 were significantly lower than those at pH 5.5. Meanwhile, these parameters did not significantly differ at pH levels 5.5–6.5 (Figs. 1, 2, and Tab. 5). This tendency showed that acidic environments (pH 5.5–6.5) were more suitable for the fry's growth. Also, the data on the fish's mean length and weight in Figures 1, 2, and Table 3 indicate that the pH groups that had significant effects on the length and weight of the fish were different between the larval stage and the fry-to-juvenile stage. That is, in the larval stage, four pH groups with significant effects on length (5.5–6.0, 6.5, 7.0, and 7.5–8.0) and three pH groups with significant effects on weight (5.5–6.0, 6.5–7.0, and 7.5–8.0) were observed. Meanwhile, in the fry-to-juvenile stage, group division was unclear; mean length and weight reached their highest values at pH 5.5, gradually decreased at the higher pH levels, and were reduced to their lowest values at pH 8. These results indicated that the fish in the larval stage were more sensitive to pH levels as compared to the fry-to-juvenile stage. Some previous authors also revealed that fish or crustaceans in early developmental stages like gametes, larvae, and juveniles would be more sensitive to acidification than adults (Whiteley, 2011; Noisette et al., 2021). The fish embryos and larvae's sensitivity to pH may be due to their high surface-to-volume ratio and their lack of specialized mechanisms in acid-base regulation (Kikkawa et al., 2003; Ishimatsu et al., 2013). It is known that the excretion process of hydrogen (H^+) ions to balance the fish body's pH occurs through the epithelia of the gills, kidneys, and intestine (Gilmour and Perry, 2009; Perry and Gilmour, 2006). Fish in early life stages are more sensitive to adverse pH conditions than in subsequent development stages, possibly because their epithelial system of the H^+ excretory organs has not yet fully developed.

4.3.2 Survival rate (SR) and Coeffcient of variation (CV)

The SR always reached its highest value at pH 6.5 and did not significantly differ from those at the acidic pH range (5.5–6.0) during the rearing period (Fig. 3). This indicated that the *C. lucius* fry lived well in the pH range of 5.5–8.0, but the better pH range was 5.5–6.5. SRs ranging from 81.74 to 90.71% in the range of 5.5–6.5 were within the SR ranges in fry of some members of the Channa genus, such as *C. punctatus* (Rahman et al., 2018; Marimuthu and Haniffa, 2006), *C. striatus* (Muntaziana et al., 2013), and *C. striata* (Ndobe et al., 2019; Helkianson et al., 2020). In addition, at equivalent acidic pH ranges to our experiment, *C. striata* juveniles also exhibited high SRs, which were 62.58% at pH 5.5–5.8 after 60 rearing days in hapa net cages (Purnamawati et al., 2018) and 92.5% at pH 5.4–6.3 after 42 rearing days in aquariums (Saputra et al., 2018). Even at lower pH levels (4.8–5.8) and in unaerated mediums, *C. striata* juveniles reached an SR of 92% after 40 rearing days in aquariums (Purnamawati et al., 2017). However, these low pH levels are still limited in the present work on *C. lucius*, and they need further research.

The CV is considered an indicator for the uniformity evaluation of individuals in the same rearing condition. The findings in CV variations (Tab. 7) supported comments that the acidic mediums were more favorable for the growth of C. lucius than the neutral to alkaline mediums in our research conditions. High CV values at high alkaline pH levels were also observed in Common carp by Heydarnejad (2012), who suggested that under the influence of alkaline pH, nutrient utilization and metabolism can explain the uneven growth in weight. Data from Experiment 2 proved that the acidic pH range (5.5–6.5) was better as compared to the neutral to alkaline pH range (7.0–8.5) for the growth and survival of *C. lucius* in the fry-to-juvenile stage.

5 Conclusion

Our findings indicated that the pH range of 5.5–8.0 was suitable for the early stage of *C. lucius*. Still, the pH range of 5.5–6.0 was better for egg incubation and the growth of larvae, while a pH range of 5.5–6.5 was better for the survival and growth of *C. lucius* in the fry-to-juvenile stage, providing evidence of the potential for the development of farming of this species in the Mekong Delta and similar regions. Future study needs are highlighted, including the successful abilities of incubation and larval and fry culture at pH < 5.5, the effects of pH variations on subsequent developmental stages, and the effects of other environmental factors as well as the usability of artificial feeds on *C. lucius.*

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