Aceh Journal of Animal Science (2019) 4(2): 80-88
DOI: 10.13170/ajas.4.2.13574
Printed ISSN 2502-9568 Electronic ISSN 2622-8734

RESEARCH PAPER

Effect of different water pH on hatching and survival rates of African catfish *Clarias gariepinus* (Pisces: Clariidae)

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Received: 30 April 2019
Accepted: 7 August 2019

ABSTRACT

Water quality parameters influence the growth and survival of different developmental stages of fishes and hence determining the optimal water quality variables is greatly important for any aquaculture farming. Among the variables, water pH is considered the key factors and plays an important role in the maintenance of the homeostasis in fishes. A study was performed to determine the impact of different level of water pH on the incubation period, hatching rate and larval survival rate of African catfish (*Clarias gariepinus*). The fertilized eggs were incubated at 28.0 ± 1.0 °C with different levels of water pH ranging from 3 -10. Twenty four pH levels were tested for incubation period and egg hatchability while 15 pH levels were tested for larval survivability in a completely randomized design with three replicates for each treatment. Fertilized and just-hatched larvae were used for this study and the effect was observed until 72 hr post-hatching. Water pH was maintained by the addition of NaOH or H_2SO_4 solutions. The incubation period of fertilized eggs was recorded to be 23.5–25.0 h at pH levels of 6.1–8.8. The increased incubation period was noticed at the rest of the acidic and alkaline pH levels. The results show that significantly highest hatching rate of eggs was recorded at the pH levels of 6.7–7.6 compared to those of lower and higher pH levels ($P<0.05$). However, no significant differences ($P>0.05$) were recognized in the hatching rates at the pH levels of 6.7 – 7.6. No hatching occurred at low pH levels between 3.1 and 3.4 and at high water pH 10. After 72 h of exposure to different pH levels, no larvae survived at pH levels below 4.5 and above 9.0. Highest larval survival (98%) was observed at pH 7.0 followed by 94% at pH 7.5 and 92% at pH 6.5. The results obtained in the present study revealed embryos and larvae can survive and tolerate in a low pH level of 3.7 and 4.5, respectively but the survival rates decreased with decreasing pH levels. For ideal hatching and the greatest larval survival of African catfish, a water pH level of 6.7-7.5 is recommended.

Keywords: African catfish, *Clarias gariepinus,* pH, hatching, larvae survival

INTRODUCTION

Physico-chemical parameters exert an immense influence on growth and survival of different stages of the fish embryo, larvae, fry, and fingerlings, and thus determining the optimal water quality variables is highly important for any aquaculture fish farming. Among the variables, water pH is considered the main factors affecting water quality. Water pH plays a major role in maintaining homeostasis in fish, and changes in pH concentrations alter the acid-base and ionic regulation and excretion of ammonia (Wood et al., 2001; Wilkie and Wood, 1994; Goss *et al.*, 1992; Wilkie *et al.*, 1993; Jensen and Brahm, 1995; Parra and Baldisserotto, 2007). For most freshwater fish species, the ideal pH value is reported to be from 6.5-9.0 (Boyd, 1990). The deviation in water pH from the normal range will adversely affect fish growth, survival, and reproduction and also causes high mortality in fish culture ponds (Zweig *et al.*, 1999). It has been

reported that the extreme pH values are toxic to fish due to ionic disequilibrium and alters the function of the circulatory system. For example, low water pH reduces the concentration of dissolved inorganic phosphorus and carbon dioxide is needed for phytoplankton during photosynthesis (Scott et al., 2005; Van Dijk *et al*., 1993). Low acidic water pH triggers degeneration of the gill tissue and increased mucus secretion that can kill fish (Boyd, 1990). High alkaline water pH inhibits the ammonia excretion and increases the ammonia level in blood plasma (Wilkie and Wood, 1996). The fluctuations in water pH away from the normal ranges are known to affect fish development and physiological conditions of fish (Zelennikov 1997; Keinanen *et al*., 2003). Low pH levels not only affect fish physiology but also indirectly affect the survival of larvae of fishes (Keinanen *et al*., 2003).

The African catfish (*Clarias gariepinus*) belongs to the family Clariidae. It is a native freshwater fish species cultivated commercially in the Netherlands, Germany, Belgium, Indonesia, Thailand, Malaysia, India, Bangladesh and Brazil (Gomaah and Naggar, 2004). It is one of the most preferred and favorite freshwater food fish species in Malaysia. They live in a variety of water bodies such as swamps, ponds, paddy fields, and rivers. They are hardy fish and can thrive in muddy, turbid and oxygen-depleted water bodies in harsh water conditions with the help of their accessory air-breathing (labyrinth) organ which allows them to breathe atmospheric air for their breathing (Haylor, 1991; Goos and Richter, 1996; Muchlisin *et al*., 2010; Asraf *et al*., 2013; Muchlisin *et al*., 2015). They rarely breed naturally under captive farm conditions, and hence artificial spawning and seed production become imperative for large scale culture of this species. The significant scale expansion of African catfish culture and farming in Malaysia is still lacking due to an inadequate seed supply of catfish. The insufficient supply of fish seed is also due to high mortalities occurring in the embryonic and larval stages.

The hatching of eggs and survival of fish larvae is influenced by several biotic and abiotic factors (Blaxter, 1974; Adebayo *et al.,* 2007). The impact of low acidic pH on embryonic fish and larval survival has been researched by several authors. (Haines, 1981; Dillon *et al.,* 1984; Jordahl, 1984; Jordahl and Benson, 1987). No fertilization at pH levels below 4.0 is noted in most fish species (Peterson *et al*., 1982), and there has also been a decrease in hatching rates (Trojnar, 1977; Menendez, 1976; Beamish, 1976). Fish species residing in extreme water pH migrate to areas where pH level is close to neutral pH for reproduction and breeding (Parra and Baldisserotto 2007). Earlier studies have been focused on the effect of acidic and alkaline pH on the hematological changes in freshwater carp species (Das *et al.,* 2006). Studies have been reported on the effects of varying temperatures on the embryonic development and larval survival of African catfish (Ajuzie and Appelbaum, 1996), salinity levels (Oladosu *et al*., 1999) and water hardness (Molokwu and Okpokwasili, 2002). There is a lack of information on pH tolerance of the embryo, hatchability, and survival of larvae of African catfish. Hence, the ideal pH concentrations for incubation, embryo hatching, and larval survival need to be determined. The aim of the present study was to evaluate the impact of water pH on the hatching rate and larval survival of African catfish (*C. gariepinus*).

MATERIALS AND METHODS

Broodstock Selection

Sexually mature adult catfish for artificial breeding and seed production were chosen from the broodfish. Based on external morphological characteristics, male and female fish were recognized (Viveen *et al.* 1985; Marimuthu *et al.* 2012). Female broodfish were differentiated by the bulged abdomen and male fish based on the elongated and pointed urinogenital papillae.

Hormone Administration and Breeding

Four female and two male brood fishes weighing between 1.0 and 1.7 kg, were chosen for the administration of hormones and spawning. Selected fishes were then acclimated in circular cement tanks (500 L capacity) for 24 hrs and fish were not offered feed during the acclimatization period. To induce artificial spawning, chosen female and male fish were administered with ovaprim

(a synthetic analog of the gonadotropin-releasing agent) (Syndel Laboratories, Canada) at the dose of 0.5 ml/kg body weight. The hormone was injected intramuscularly at the dorsal side of the fish. The hormone injected fishes were kept separately in cement tanks.

After a 12 hr of the latency period, eggs were stripped from the female fish manually by giving a slight pressure on the abdomen. Testes were removed from male fish, and sperm was pressed into sterile dry Petri plates. Eggs collected from the four females were pooled together, and milt from two male was added and mixed gently. Fertilization was initiated by adding chlorine-free tap water to the egg and milt mixture. This mixture was held for about 2 minutes after which the eggs were rinsed twice before incubation to remove surplus milt. For the embryo hatchability study, fertilized eggs were instantly put in experimental plastic aquariums. In order to acquire hatchlings for larval research, the remaining fertilized eggs were introduced into the glass aquarium (100 L capacity).

Experimental Design

For embryo hatchability, 24 different pH levels viz., 3.1, 3.4, 3.7, 4.0, 4.3, 4.6, 4.9, 5.2, 5.5, 5.8, 6.1, 6.4, 6.7, 7.0, 7.3, 7.6, 7.9 8.2, 8.5, 8.8, 9.1, 9.4, 9.7 and 10.0 were tested. The acidic and alkaline pH concentrations were maintained with the addition of 1 % stock solution for sulphuric acid and 0.2 % stock solution for alkaline sodium hydroxide and the stock solution was prepared as described by Kugel and Peterson (1989) and Lopes et al. (2001). This study was conducted in 5 L circular plastic aquaria. Two hundred fertilized eggs were placed in each aquarium and three replicates were maintained for each pH level. Aeration was provided throughout the study using aquarium air stones, and the water was not exchanged during the experimental duration.

Water quality characteristics of experimental used waters were determined according to APHA (1985). A digital pH meter was used for measuring pH. The mean water quality parameters of the experimental used water were as follows: temperature 28.0 ± 1.0 °C, pH 7.2 ± 0.2, dissolved oxygen 6.5 ± 0.1 mg/L, hardness 35.0 ± 5.2 mg/L, total alkalinity (as of $CaCO_3$) 29.0 ± 1.0 mg/L, ammonia 0.01 ± 0.001 mg/L, nitrite 0.01 ± 0.001 mg/L, nitrate 2.7 ± 0.1 mg/L, and chloride 6.0 ± 1.0 mg/L. All water quality parameters were measured at the beginning and end of the experiment. Dead eggs were counted and removed at four hrs intervals to prevent any fungal growth. Mortality and hatching rates were recorded throughout the experimental period. Eggs were considered as dead when the eggs turned into opaque and became pale white. Hatching was defined as the rupture of the egg membranes by the tail region. The hatching rate was determined as the proportion of hatched eggs to a total number of fertilized eggs in each aquarium. The incubation period was recorded as the time duration difference between fertilization and the hatching of eggs.

To assess the effect of water pH on the survival of hatchlings, 15 pH levels were chosen within the acidic and alkaline pH ranges. The pH values used were 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0 in a completely randomized design with three replicates for each pH level. This study was conducted in 5 L circular plastic aquaria, and each aquarium was stocked with 200 newly hatched hatchlings. The pH values were evaluated twice daily with a digital pH meter at10.00 and 17.00 hrs and the pH concentrations were maintained by adding NaOH or H_2SO_4 solutions.

All aquaria were continuously supplied with aeration, and water was not exchanged during the experiment. Mortality of larvae in each aquarium was checked every 12 hrs after the beginning of the experiment until 72 hours. Dead larvae were recorded and removed, and then fixed in 4.0% formalin for microscopic observation. Non-motile, enlarged and white opaque larvae were considered as hatchling mortality.

Data Analysis

The data collected from each pH level for mean hatching and larval survival were evaluated using one-way analysis variance (ANOVA) followed by Duncan's multiple post hoc tests. The *P*-

value < 0.05 was considered significant difference. All statistical data analyses were performed using SPSS version 20.

RESULTS

The effects of water pH on the incubation period and hatching rate of fertilized eggs are shown in Fig. 1 and 2. A significant difference was noticed between incubation times of catfish eggs exposed to different pH levels ($P<0.05$). The incubation times of fertilized eggs were determined as 23.5 to 25.0 hrs from the pH levels 6.1 – 8.8. In the rest of the acidic and alkaline pH levels, the increased incubation time was noticed. The greatest hatching rate ($P<0.05$) was noted at 6.7–7.6 pH levels compared to the rest of the pH concentrations (Figure 2). The hatching rate was reduced at all the lower and higher pH ranges (Figure 2). It was also found that no eggs were survived at the pH values of 10 and 3.1–3.4. At these pH levels, embryonic development was not progressed, and all the embryos died and subsequently turned to pale white color within 10–30 min of exposure.

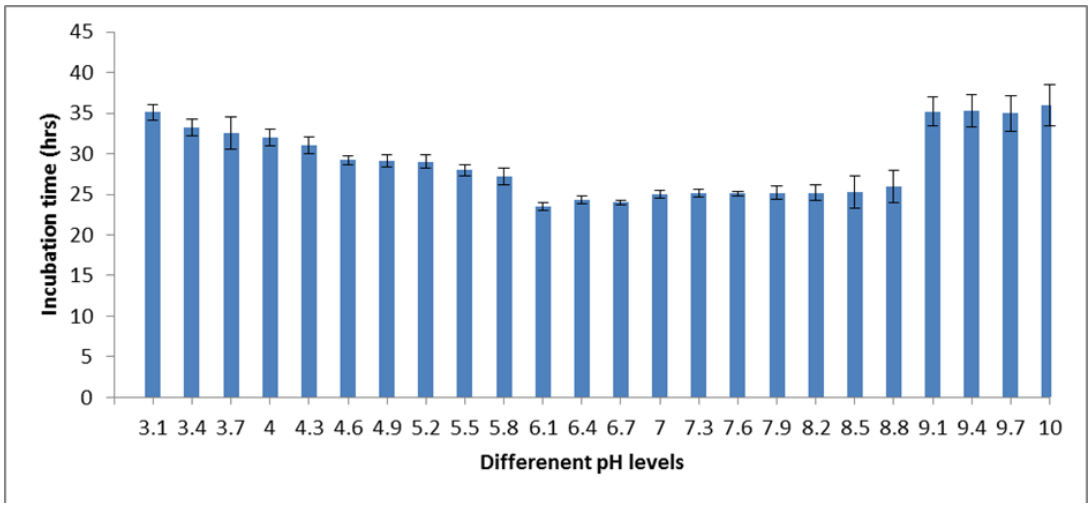

Figure 1. Effect of different pH levels on the incubation time (hrs) of *C. gariepinus*

Few hours before hatching of eggs, the embryos showed the twisting movement inside eggs. The egg membrane was ruptured with the embryo's caudal region at 24 hrs after fertilization and the tail arose first followed by the remainder of the body. For a few hours after hatching, the body of the freshly hatched embryo stayed in a bent position, with the head bending over the yolk. The newly hatched embryo was transparent, light yellowish, and lacking pigmentation. However, in the present study, low embryo survival rates were reported at both low acid and elevated alkaline pH, suggesting that fertilized eggs and early catfish embryos and larvae were under stress at these pH concentrations.

High mortality in African catfish larvae was noted, subjected to extreme pH levels, particularly at acidic pH. After 4 hours of exposure, 100% mortality was observed at pH concentrations below 4.5. In the larval experiment, 100% larval mortality was observed after 72 hours of exposure at pH concentrations 3.0 – 4.5 and this outcome shows that catfish larvae are extremely susceptible to pH values below 4.5. Larvae exposed to pH levels from 5.0 to 6.0 showed a survival rate of 24–64 %. All the larvae exposed to alkaline pH levels (9.0, 9.5 and 10.0) were dead at 24 hrs of exposure. The highest survival (90%) and above was noticed at 6.5 – 7.5 pH levels. The survival rate of catfish larvae decreased progressively at 8.0 and 8.5 pH levels.

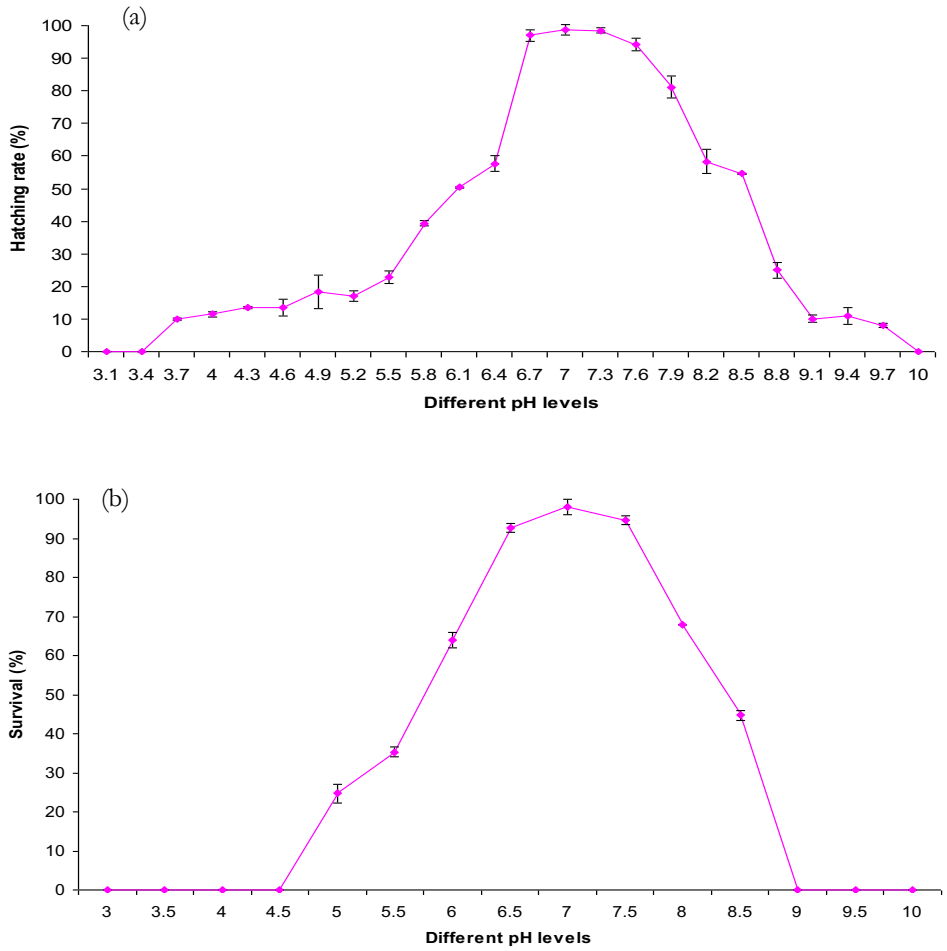

Fig. 2. (a) Effect of different water pH levels on the hatching rate of *C. gariepinus*. (b) Effect of different water pH levels on the survival of larvae of *C. gariepinus*. Each value is the mean of three replicates and the standard deviation.

DISCUSSION

The results revealed that pH had a marked effect on the incubation period and the time required for the embryonic development to complete and hatching to occur. Various findings were recorded when embryos in different fish species were incubated with different pH concentrations. Rask (1983) reported that the incubated *Perca fluviatilis* eggs at water pH 4.0 showed an increased period of incubation compared to the normal neutral pH exposed eggs. Several researchers have observed comparable findings on incubation period at the low in zebrafish (*Brachydanio rerio*) by Johansson *et al.* (1973); perch (*Perca fluviatilis*) by Runn *et al.* (1978); brook trout (*Salvelinus fontinalis*) by Swarts *et al.* (1978). Peterson et al. (1980) reported that the incubation period of Atlantic salmon (*Salmo Solar*) eggs were independent of pH up to 4.5 when they raised in acidic water pH from fertilization, however hatching was delayed when transferred from neutral pH after eye pigmentation stage. Increased incubation period reported due to acid stress in other fish species like Fathead minnow (*Pimephales promelas*) (Mount, 1973), Brown trout (*Salmo trutta fario*) (Brown and Lynam, 1981) and brook trout (*Salvelinus fontinalis*) (Swarts *et al.*, 1978). Trojnar (1977) and Menendez (1976) recorded the shorter incubation period of eggs at low pH in *Salvelinus fontinalis*. The sensitivity of the fish embryo to low pH concentration was demonstrated in brook trout and

salmonids (Menendez, 1976; Trojnar 1977; Huisman *et al.* 1983; Lacroix *et al.* 1985). It has been elucidated that the increased incubation period is due to an impairment of the hatching process due to the activity of the hatching enzyme at lower pH. Both chorionase activity and embryo activity are inhibited at low pH, delaying hatching time and inhibiting hatching (Haya and Wainwood, 1981; Peterson and Martin Robichaud, 1983).

At pH concentrations of 6.7–7.6, the greatest hatching rate was noted at lower and higher pH concentrations. The reduced hatching rate was observed in all the lower and higher pH ranges. It was also found that no eggs can survive at the pH values of 10 and 3.1–3.4 and all the embryos died within 10–30 min of exposure. Nevertheless, hatching occurred at a wide range of pH levels from 3.7-9.7. This information also shows that catfish eggs are more resistant than other fish species to acidic and alkaline pH stress. Several authors have indicated that in acidic conditions no hatching was observed (Haya and Wainwood, 1981; Peterson and Martin Robichaud, 1983). At the pH levels of 9.1–9.7, and below 5.8, 70–90 % of the eggs died within 2–4 hrs before reaching to the 4–8 blastomere stages. The study observed that the high fish embryo mortality was observed at the first 4 hr of exposure to acidic and alkaline pH levels. During the first 10 hours, the mortality rate at lower pH concentrations was greater, while it was lowered after 15 hours of exposure, showing that eggs are prone to acid exposure instantly after fertilization until the membranes are hardened. After egg hardening, a protective resistant barrier is developed by the chorion and plasma membrane to protect the developing embryo from external media. For other species of fish such as *Cyprinus carpio* (Oyen *et al.*, 1991), *Stizostedion vitreum, Catostomus commersoni, Coregonus clupeaformis, and Notropis cornutus* (Holtze and Hutchinson, 1989), similar observations have also been recorded. Cykowska and Winnicki (1972) reported the strength of the egg membrane increased 7 times in the first 12 h after fertilization and 15 times after 24 h. Johansson and Milbrink (1976) reported the embryonic development of roach and perch stopped at a water pH below 4.6. Conversely, few studies have reported the tolerance of fish eggs to alkaline pH. Krishna (1953) found that the eggs and Alevins of trout showed mortalities at pH values over 9.0. Generally, the water pH ranges from 5.0 to 9.0 is considered harmless to fish.

The research found low embryo survival rates at low acidic and elevated alkaline water pH, showing that fertilized eggs and early embryos larvae of catfish were under stress at these pH concentrations. African catfish larvae, subjected to extreme acid pH values, the greater mortality was reported. Norrgren and Degerman (1992) revealed that the Atlantic salmon (*Salmo salar*) was susceptible to low pH levels, with 100% larval mortality at pH 5.1. Jezierska and Witeska (1995) also noted 100% larval mortality at pH 5.5 in common carp (*Cyprinus carpio*).

CONCLUSION

It is concluded that embryos and larvae could survive and tolerate at a low pH level of 3.7 and 4.5, respectively, but the survival rates decreased with decreasing pH levels. Therefore, a water pH level of 6.7 – 7.5 is highly recommended for optimal hatching and larval survival for African catfish seed production and hatchery management.

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