Effects of pH on Fertilization and the Hatching Rates of Far Eastern Catfish *Silurus asotus*

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Abstract

In this experiment, we examined the survival, fertilization, hatching times, and hatching rates of Far Eastern catfish *Silurus asotus* at pH ranging from 2 to 13 under laboratory conditions. Eggs could be fertilized at pH 3-12. In a hatching experiment, mortality was first observed at pH 13, when all fertilized eggs died within 8 min, followed by pH 2 (30 min), pH 12 (60 min), pH 3 (4 h), and pH 11 (5 h). Hatching only occurred at pH 4-10, with the highest hatching rate at pH 7 (52%) and the lowest at pH 10 (24%). Hatching rates in acid solutions were higher than in alkaline solutions, although the difference was not significant. Hatching was first observed at pH 10, beginning 27 h after fertilization and ending at the 31 h. A clear difference was observed between hatching times, ranging from 31 to 64 h and increasing in order with descending pH.

Key words: pH, Fertilization rate, Hatching rate, *Silurus asotus*, Far Eastern Catfish

Introduction

pH is the main factor affecting water quality. Extreme pH negatively affects fish growth and reproduction (Zweig et al., 1999) and even causes massive mortalities in fish culture. Sensitivity to extreme pH conditions varies according to fish species and age, with fish showing lower tolerance at the embryonic and larval stages (Lloyd and Jordan, 1964). The fertilization of most fish species is unsuccessful at water pH lower than 4.0 (Peterson et al., 1982). The fertilization success of Atlantic salmon *Salmo salar* decreased to zero with a decrease in pH from 5.0 to 4.0 (Daye and Glebe, 1984), and an increase in salmonid egg mortality due to fertilization in acidic water (pH 4.6) was also found by Carrick (1979) and Parker and McKeown (1987). Relatively little is known about the effects of alkaline discharges on the fertilization and hatch rates of fish, which may reflect the lesser importance of the problem. However, in cases in which high pH is caused by the vigorous photosynthetic activity of aquatic plants, accompanying high temperatures and supersaturation of dissolved gases (together with other factors) may also contribute to fish mortality to a

greater or lesser extent, making a correlation between mortality and pH data alone quite difficult.

Generally, the effect of low pH on fish fertilization and hatching is not often a subject of research (Sayer et al., 1993) except in salmonids. Many studies have been made on the effects of acidification on the fertilization and hatching of salmon and trout, but such studies on Far Eastern catfish *Silurus asotus* had not been conducted. Therefore, in this study, we investigated the effects of a wide range of pH on the mortality, fertilization, and hatching rates of catfish eggs.

Materials and Methods

Preparation of pH solutions

The water used was groundwater with a pH of 7.34, which also served as the control water. The study was conducted at the aqua farm of Kunsan National University. pH solutions

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ranging from 2 to 13 were adjusted using sodium hydroxide and hydrochloric acid. Five milliliters of pH solutions was added to Petri dishes for the observation of fertilization. For the hatching experiment, 200 mL of pH solution was added to a 400-mL beaker, and solutions were aerated with an air pump to ensure sufficient dissolved oxygen (above 7 mg/L). Temperature was maintained at $23.5 \pm 1^\circ\text{C}$ and pH was measured with a Mettler Toledo MP-220 (Mettler-Toledo GmbH, Switzerland). pH was tuned every hour and the solutions were wholly changed every 6 h during all experimental periods. Fertilization and hatching experiments were performed in triplicate for each pH level.

Egg and sperm collection

The experiment was performed at the aqua farm of Kunsan National University. Three-year-old brood fish were selected and kept isolated in holding tanks. Spawning was induced in female brood fish using intraperitoneal injections of human chorionic gonadotropin at the rate of 10,000 IU/kg body weight. These fish were then stocked for 12 h at a water temperature of $28 \pm 0.5^\circ\text{C}$. Twelve hours later, eggs were stripped into a dry bowl. Sperm were obtained through surgical removal of the testis. Adherent tissue was dissected away and the testes were pooled in physiological saline. Some of the stripped eggs were fertilized immediately with milt after sperm activation was initiated by the addition of water and stocked in an incubator. Sperm from one male were used to fertilize eggs from one female. The remaining eggs were stocked in a dry bowl and sperm were stocked in physiological saline for the fertilization experiment.

Fertilization and hatching rates

To observe the fertilization rate, 50 eggs were placed into the prepared 5 mL pH solutions; sperm were injected into the dish and mixed equably, and after about 1 min of gentle stirring, fertilization could be checked under a dissection microscope (10–100 $\times$ magnification). Fertilization membranes formed immediately when an egg was fertilized so we could use them as a standard to determine whether such an event occurred. Fertilization rates were calculated as the number of total eggs divided by the number of fertilized eggs.

To determine the hatching rate, 50 fertilized eggs were selected from the incubator and placed randomly into the 200 mL prepared pH solutions. Mortalities and hatching rates were then recorded during the experimental period. Eggs were considered dead when parts of the content turned opaque and white. Dead eggs were counted and removed to prevent fungal growth. Hatching was defined as the rupture of the egg membranes by the tail. The hatching rate was determined as the proportion of hatched eggs to total eggs. Hatching time was recorded as the time span between fertilization and the hatching of the last egg.

Fig. 1. Fertilization rate at different pH levels. Mean $\pm$ SD with different letters are significant different based on ANOVA ($P < 0.05$).

Statistical analysis

Data were analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to compare the mean values at different pH levels. $P < 0.05$ was considered to indicate a significant difference.

Results and Discussion

In this experiment, catfish eggs were fertilized at pH 3–12, as shown in Fig. 1. Fertilization rates from pH 4 to 9 were above 70% and obviously higher than those at pH 3, 10, 11, and 12. The fertilization rates generally declined with decreasing acid concentrations and increasing alkalinity concentrations. No fertilization occurred at pH 2 or 13. The decrease in fertilization success may have been due to the effects of acidic water either on spermatozoa motility or on the eggs. We observed that egg membranes dissolved at pH 2, although at pH 13, eggs became opaque and white in a few minutes; we, however, did not notice the motility of spermatozoa. According to Daye and Glebe (1984), the motility time of Atlantic salmon spermatozoa decreased from pH 7.0 to 4.0, around which no motility was found after a lag of 20 s. The proportion of motile spermatozoa of brook trout decreased as the pH was reduced from 5.0 to 3.5 and completely ceased at pH 3 (St-Pierre and Moreau, 1987). Similar findings were reported for alkaline conditions. The sperm of common carp had a lower period of motility when the pH value of the water was raised to between 8.2 and 9.5, and pH values above 9.0 were found to be lethal (Elster and Mann, 1950). In our experiment, eggs could be fertilized at pH 3–12. This was a considerably wide range compared to other fish species, suggesting that catfish eggs were more tolerant of extreme pH conditions. However, in pH 12, 11, and 3 solutions, the fertilized eggs did not survive long, all dying within 1 h, 5 h, and 4 h, respectively.

The survival rates of fertilized eggs at different pH levels are shown in Fig. 2. Mortality was first observed in pH 13 solutions, with all eggs dying within 8 min. At pH 2, embryonic

Fig. 2. Survival rate at different pH levels.

Fig. 3. Hatching rate at different pH levels. Mean $\pm$ SD with different letters are significant difference based on ANOVA ($P < 0.05$).

Fig. 4. Hatching time at different pH levels.

development stopped completely and all eggs died within 30 min. Cleavage occurred at pH 12 and some eggs developed to the 2-cell stage, but all eggs died in 1 h. Eggs started dying 60 min after fertilization in pH 3 solutions. Two-thirds of the eggs died before entering the 4-cell stage, and all eggs died within 4 h. At pH 11, most eggs died before the morula stage and all died within 5 h postfertilization. Mortality was high in the first 15 h in these solutions, after which time the death rate became low. The period of sensitivity to acidity and alkalinity appeared to be the first 5 h after fertilization. This phenomenon has also been reported for other species of fish such as *Cyprinus carpio* (Oyen et al., 1991), *Stizostedion vitreum*, *Catostomus commersoni*, *Coregonus clupeaformis*, and *Not-*

ropis cornutus (Holtze and Hutchinson, 1989). Mortality in pH 4-10 solutions was higher during the first 15 h and became low thereafter, indicating that eggs are susceptible to acid exposure immediately after fertilization until the membranes have hardened. Cykowska and Winnicki (1972) reported that egg membrane strength increased sevenfold in the first 12 h after fertilization and had increased by 15 times after 24 h. After hardening, the chorion and the plasma membrane form a resistant barrier to protect the developing embryo from external media.

Hatching rates are shown in Fig. 3. Hatching occurred at pH 4-10, but the hatching rate was low, even in the control with the highest hatching rate of 52%, followed by pH 6, pH 5, pH 8, pH 4, pH 9, and pH 10. No obvious difference was seen between the control and pH 4, 5, 6, or 8 solutions, but control rates were significantly higher than those in pH 9 and 10 solutions. Hatching rates in acid solutions were higher than those in alkaline solutions. In fact, this was supposed to be a wide pH range for hatching compared to other fish species. Many reports of hatching failure under such acidic conditions have been published. For example, Trojnar (1977) reported that deformations and death of white sucker *C. commersoni* embryos occurred at pH 5.0. Mount (1973) found that deformations of fathead minnow *Pimephales promelas* eggs took place at pH 5.9. Johansson and Milbrink (1976) reported that the embryonic development of roach and perch stopped at a pH lower than 4.6.

However, fewer reports have described the tolerance of fish to alkaline solutions, and generally, the pH range of 5-9 is accepted as harmless to fish. Krishna (1953) found that for trout eggs and alevins, mortalities occurred above a pH value of 9.0, but the length of exposure was not given. The various developmental stages of turbot eggs showed different sensitivities to alkaline waters, with the most sensitive stage being that of embryo segmentation, when a pH value of 8.0 killed half of the eggs (Volodin, 1960). Resistance increased after this stage, but even at pH 9.0, hatching was delayed. This was different from the results of our experiment, wherein eggs hatched in alkaline solutions, even at pH 10, with a hatching rate of about 24% (Fig. 3), followed by pH 9 (35%) and pH 8 (48%). These data suggested that catfish eggs were less sensitive to acid and alkaline stress compared to those of the above-mentioned fish species.

A significant difference was observed between hatching times, as shown in Fig. 4. Hatching time increased with decreasing pH values. Although the hatching rate was lowest at pH 10, the hatching time was the shortest, with hatching beginning 27 h after fertilization and ending at the 31st h. The eggs in pH 4 solutions did not start hatching until 53 h after fertilization, and 64 h were required for all eggs to hatch. In this experiment, hatching time was longer than those recorded under field conditions, which may be attributable to experimental conditions being less stable than field conditions. The eggs in our experiment seemed to suffer more artificial stress

from factors such as pH adjustment and water change. The delay in hatching time, as observed in catfish, has been frequently noted in eggs of other fish species. Rask (1983) found that eggs of *Perca fluviatilis* reared at pH 4 showed a delay in hatching time relative to that of the control. Delays in hatching time resulting from acid stress were also found for *P. promelas* (Mount, 1973), *Salmo trutta fario* (Brown and Lynam, 1981), and brook trout *Salvelinus fontinalis* (Swarts et al., 1978). This may imply that eggs in acidic water were vulnerable to predation for a longer period than eggs in neutral water. However, contradictory observations have even been reported for salmon species. For example, Daye and Garside (1979) found no influence of acid stress on the rate of development of Atlantic salmon at pH 6.8-3.7. Menendez (1976) also found no such effects for *S. fontinalis*. Trojnar (1977) even recorded more rapid development of *S. fontinalis* at pH levels lower than 5. However, in this study, hatching time in alkaline solutions was faster than those in the neutral and acid solutions, especially under pH 10, when hatching occurred in only 31 h. Few reports exist on the hatching times of eggs under alkaline conditions, and this accelerated hatching was first observed in the present experiment. However, the reason for it remains unclear, and further study is still needed in this field.

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