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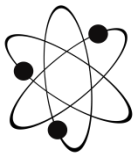

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Removal of Eggs Adhesiveness of African catfish (*Clarias gariepinus*) at Different Concentrations of Urea Solution

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Abstract –The objective of the present study was to determine the best concentration of urea solution and rinsing time to remove adhesiveness of African catfish eggs. Four different concentrations of rinse solution were used in this experiment comprising of 2 g, 4 g, 6 g and 8 g of urea diluted in 4 g NaCl/L of water producing a concentration of 0.486 mM, 0.973 mM, 1.458 mM, and 1.946 mM of urea concentration respectively, while 4 g NaCl/L of water without urea were used as control. The fish eggs were rinsed with the solutions at different durations namely; 1, 5 and 10 minutes. Each rinsing solution concentration and rinsing time were conducted in three replicates. The fertilization and hatching rates increased and peaked from the control (0 mM of urea) to 0.466 mN urea and decreased when concentration of urea was increased to 0.973 mM urea and 1.458 mM urea, then increased again at 1.946 mM urea, but these values were lower than at 0.486 mM urea. Thus, the best concentration for fertilization and hatching rates were 0.486 urea of urea. However 0.973 mM of urea was optimum in reducing clumping of eggs.

Keywords: Clumping; Fertilization; Hatching; Brood fish

Introduction

The African catfish, *Clarias gariepinus* is considered as one of the most economically important freshwater fish in Malaysia and is a source of high quality protein in developing countries. This species is known for its resistance to diseases, high growth rate, resistance to handling and stress, ability to tolerate a wide range of environmental parameters and high stocking densities under culture conditions and high meat quality (Rasowo *et al.*, 2007; Wachirachaikarn *et al.*, 2009). Since the last 30 years, they have been introduced for aquaculture in many European and South Asia countries (Tripathi, 1996). This fish has many advantageous characteristics for aquaculture; it is easily induced for breeding activity in the hatchery, it can survive outside the water for a long period of time and can crawl on land during the drought season, perhaps to other water bodies.

However, there are some problems in producing African catfish larvae. One of the significant problems is low hatching and survival rates (Muchlisin *et al.*, 2010). This problem is probably due to the adhesiveness of eggs where the eggs clump to each other when they are released into the water, and thus resulting in low fertilization and hatching rates (El-Gamal and El-Greisy, 2008). In addition, adhesiveness of eggs can cause high larval mortality (Abigail *et al.,* 2010).

One of the ways to solve this problem is to rinse the eggs with certain solutions for example, urea, mud, milk, colin etc, the solutions are sometimes species specific. For example, mud solution has been utilised to rinse the eggs of the Japanese dace, *Triborodon hakonensis* (Nakamura, 1966), river silt in white sturgeon, *Acipenser transmontanus* (Doroshoft *et al.*, 1983) and milk powder in carp, *Cyprinus carpio* (Soin, 1976). Urea solution has been used in carp, *Cyprinus carpio* (Woynarovich, 1962; Rothburd, 1978). In addition, tannic acids have also been used as rinsing agent in himri barbel, *Barbus luteus* (Al-Hazzaa and Hussein, 2003) and enzymes (α-Chymotrypsin (EC 3.4.21.1. MERCK) and Alcalase DX (PLN 04715)) in the common carp (Linhart *et al.*, 2003).

To date, no study has been done to remove adhesiveness of the African catfish eggs urea solution. Therefore, this study is important and significant to find the best solution to eliminate the stickiness of the African catfish eggs so that fertilization and hatching rates could be increased. Herein, urea solution at different concentrations was evaluated for African catfish eggs. The objective of the present study was to determine the best concentration of urea and rinsing time to remove adhesiveness of African catfish eggs.

Materials and Methods

Brood stock

Two males and two females of the African catfish (*Clarias gariepinus*) weighing approximately 2-3 kg were selected and kept in a tank seperately at the Aquaculture Research Centre, Universiti Sains Malaysia. The fish were injected with a single injection of ovaprim (Syndel Laboratories Ltd. Qualicum Beach, BC Canada) at a dose of 0.5 ml/kg body weight to stimulate sperm and egg maturation. Males and females fishes were kept in the separate tanks with aeration under room temperature.

Rinse solution preparation

Four different concentrations of rinse solution were used in this experiment namely; 2 g (0.486 mM), 4 g (0.973 mM), 6 g (1.458 mM) and 8 g (1.946 mM) of urea in 4 g NaCl/L of water, and 4 g NaCl/L of water without urea was used as control. The fish eggs were rinsed with the solutions at different duration periods namely for; 1, 5 and 10 minutes. Each rinsing solution concentration and rinsing time were conducted in three replicates.

Sperm and egg collections

Approximately 12 hours after the injection of ovaprim, both female and male fish were anesthetized with three drops of clove oil extract dissolved in 10 liters of tap water prior to sacrifice by spinal transaction. Then the testes were removed by abdominal dissection. These were then cleaned with a towel and semen was gently squeezed out and collected in two 50 ml beakers which were kept on ice in a styrofoam box (4 ^{o}C). The females were squeezed abdominally to collect the eggs and placed in a petri dish. The sperm and egg were fertilized in the petri dish and gently mixed with feather with 3-5 drops of filtered tap water added.

Rinsing procedure

The fertilized eggs were collected in 1ml aliquots using a 5 ml syringe and placed in a 50ml beaker for each treatment. Then approximately 4ml of rinsing solution was added into each beaker and gently stirred, and then left for 1, 5, 10 minutes, respectively in the experimental beakers (in three replicates). After completion of each duration period, the samples were incubated in 1L plastic jars with filtered tap water and aerated continuously under temperature of 27°C.

Clumping, fertilization and hatching rates examination

Three parameters were determinated to assess the success of this experiment; (a) egg clumpness, (b) fertilization rate and (c) hatching rate. The success of resolving the egg adhesiveness was indicated by the lower percentage of clumping eggs. This is calculated by the number of clumping eggs divided by the total number of incubated eggs. The percentage clumping eggs were recorded 15 minutes after incubation. The success of fertilization was evaluated after 30 minutes of incubation. The fertilized eggs were evaluated by the development of transparent embryos in contrast to the white color of unfertilized or dead eggs. Hatching rates were observed after 24 hours of incubation, by counting the number of larvae produced.

Data analysis

The data of fertilization, hatching and clumping rates at different concentrations of urea and duration of rinsing were tested by the multivariate general linear model to examine the variables namely concentrations of urea, and rinsing times as the main effect, and its interactions on the clumping, fertilization and hatching rates. Duncan's multiple range tests was used to determine the best concentration and rinsing time.

Results and Discussion

The ANOVA tests showed that there were significant effects of treatments on the clumping, fertilization and hatching rates ($P<0.05$). The clumping rate ranged from 6.18% to 23.57% in control (0 mM urea) with 1 minute rinse time and in 8 g (1.946 mM) urea with 5 minute rinse time, respectively. The fertilization rate ranged from 12.19 % to 38.35% in 6 g (1.458 mM) urea with 5 minutes rinse time and in 2 g (0.486 mM) urea with 1 minute rinse time, respectively. In addition, the hatching rate ranged from 4.12% to 25.45% in 8 g (1.946 mM) urea with 5 minutes rinse duration and in 2 g (0.486 mM) urea with 1 minute rinse duration time (Table 1). Among the treatments (i.e. excluding the control), the 4 g (0.973 mM) urea with 10 minutes rinse time gave the best results for lowering of clumping rate, but this value was not significantly different from 4 g (0.973 mM) urea with 1 minute rinse time. On the other hand, the 2 g (0.486 mM) urea with 1 minute rinse time treatment showed the highest fertilization. However, it was not significantly different from 8 g (1. 946 mM) urea with 1 minute rise time, while for hatching rate the best result was found at 2 g (0.486 mM) urea with 1 minute rinse time.

The present study has indicated that the adhesive nature of the African catfish egg membrane was not effectively reduced by urea, instead lower clumping of eggs was observed in the control (without urea). A contrasting result was reported by Rothburd (1978) and El-Gamal and El-Greisy (2008) in the carp. They reported that urea effectively removed stickiness of carp eggs and enhanced the fertilization and hatching rate. The difference observed in this study, might be due to the unique adhesion apparatus on *Clarias gariepinus* eggs as reported by Riehl and Appelbaum (1991) and urea may not be the best agent to resolve this. However, the highest percentage of fertilization and hatching were found in rinsing agent of 2 g urea with 4 g of NaCl/L (0.486 mM urea) with 1 minute rinsing time, although there was no significant difference with other treatments. The urea functions to remove glutinosity of egg (Woynarovich and Woynarovich, 1962; Rothbard 1978). However, at higher concentrations the urea may cause the basal membrane of the egg to be overly abraded and disturb the chorion development resulting in lower fertilization

and hatching rates. Similar observation was reported for tannic acid treatment used in the pikeperch, *Sander lucioperca* eggs. When high concentration of tannic was used it caused the chorion to harden, thus decreasing the chorionase activity (Demska-Zakęś *et al.*, 2005). This is in agreement with the present findings that the fertilization and hatching rates were decreased as urea concentration increases in the rinsing agent.

Table 1. Mean ±SD percentages of clumping, fertilization and hatching rate based on interaction effects between time of rinsing and rinsing agent. The mean values in the same column with different superscipt is significantly different (P>0.05).

Rinsing Agent	Time (Minute)	Clumping rate (%)	Fertilization rate (%)	Hatching rate (%)
0 g urea + 4 g NaCl/L water (0 mM urea as control)	1	6.18 ±2.91^{a}	29.21 ±26.48^{b}	15.32 ±13.64^{b}
	5	12.63 ±5.94^{b}	14.43 ±8.78^{a}	9.05 ±10.70^{a}
	10	19.62 ±8.28^{bc}	21.15 ±34.53^{ab}	15.59 ±27.00^{b}
2 g urea + 4 g NaCl/L water (0.486 mM urea)	1	15.23 ±4.83^{b}	38.35 ±42.02^{cd}	25.45 ±29.97^{bc}
	5	14.96 ±2.50^{b}	33.06 ±32.65^{c}	14.61 ±15.11^{ab}
	10	16.40 ±14.05^{b}	20.25 ±16.62^{ab}	13.98 ±12.61^{ab}
4 g urea + 4 g NaCl/L water (0.973 mM urea)	1	11.83 ±3.98^{ab}	32.71 ±39.07^{c}	12.99 ±12.92^{ab}
	5	17.11 ±6.85^{b}	22.94 ±17.91^{ab}	11.11 ±10.09^{ab}
	10	10.22 ±7.22^{ab}	18.01 ±15.59^{a}	10.48 ±9.14^{ab}
6 g urea + 4 g NaCl/L water (1.458 mM urea)	1	22.13 ±5.17^{c}	29.57 ±31.85^{b}	19.71 ±25.33^{b}
	5	18.10 ±5.65^{b}	12.19 ±8.26^{a}	6.09 ±4.38^{a}
	10	14.25 ±15.42^{bc}	19.18 ±14.83^{ab}	10.13 ±10.81^{ab}
8 g urea + 4 g NaCl/L water (1.946 mM)	1	19.27 ±4.32^{b}	38.26 ±36.29^{cd}	11.20 ±9.03^{ab}
	5	23.57 ±7.18^{c}	16.22 ±14.29^{a}	4.12 ±3.92^{a}
	10	16.67 ±5.82^{b}	33.87 ±28.35^{c}	13.71 ±16.48^{ab}

The present study indicates that the optimal time needed to rinse the African catfish eggs was one minute. The findings showed that the fertilization and hatching rates were high and clumping rate was lowest when the eggs were rinsed for 1 minute. A contrasting recommendation was proposed by Demska-Zakęś *et al.* (2005), in that the minimum time needed to rinse the eggs of pikeperch, *Sander lucioperca* must be more than 2 minutes, even if using the highest concentration of rinsing agent. This might be due to the swelling process. When the eggs are immersed too long in the rinsing agent, the egg size decreases or the eggs may even disrupt due to the osmotic pressure (Demska-Zakęś *et al.*, 2005).

Overall, the fertilization and hatching rates recorded in this study were lower compared to studies reported by Molokwu and Okpokwasili (2002), Adewumi *et al.* (2005) and Ndimele and Owodeinde (2012). The success of a breeding program is determined by various factors. The quality of eggs and sperm are two of the important factors in a breeding program. There are several factors that influence the production and quality of seed quality of brood stock (Marteinsdottir and Steinarsson, 1998; Al-Hazzaa and Hussein, 2003), by incubation condition and other physiological influences (Schreck *et al.*, 2001; Al-Hazzaa and Hussein, 2003). It is very likely that the quality of eggs and sperms in this study was sub-optimal and therefore resulted in lower fertilization and hatching rates. The brood stocks were collected from local farmers and had been stocked for a long time in the selling tanks. They had therefore been subjected to stressful conditions at very high stocking density and irregularly fed. This could have resulted in the production of low quality eggs or sperms.

The use of a physical approach in the form of rinsing agent such as powdered milk, kaolin and talc to coat the eggs, eliminates or at least reduces the aggregation when the eggs are released into the water (Soin 1976; Schoonbee and Brandt 1982) providing more satistifactory results than using urea. Khan *et al.* (1986) reported that using milk as the rinsing agent to remove adhesiveness gave better results than using urea in the common carp, *Cyprinus carpio* eggs (Linhart *et al,* 2003). However, this study has contributed new practical information for the local farmers, but the results need to be further investigated and clarified by working on good quality brood stocks in optimal conditions. For instance, the brood stock should be reared in a special tank (brood stock tank) and fed with high quality feed prior to be used for study. Therefore, this study is considered a preliminary investigation and a starting point for further similar studies.

Conclusions

The present study revealed that the concentration of 2 g urea + 4 grams NaCl/L of solution (0.486 mM urea) with one minute rinsing time gave the highest fertilization and hatching rates and lower clumping rate. However 0.973 mM of urea was optimum in reducing clumping of eggs Further investigation with better quality stocks should be conducted in future to test the effectiveness of urea as a rinsing agent for the African catfish eggs.

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