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Effects of Different Treatment Substances and Incubation Conditions on the Hatching Performance of Fairy Shrimp (*Streptocephalus* sp.) Cysts

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ABSTRACT

A study on improving the hatching quality of fairy shrimp (*Streptocephalus* sp.) cyst hatching was performed. The chemicals used were H_2O_2 at doses of 3, 5, and 7%, as well as $NaHCO_3$ (baking soda) at doses of 10, 15, and 20g, to study the effects of these substances. In a separate experiemnt, the cysts were hatched either with or without aeration after determining the optimal concentrations of H_2O_2 and $NaHCO_3$ to stimulate cyst hatching. The results showed that H_2O_2 improved the fairy shrimp cyst hatching rate at a dose of 3% and $NaHCO_3$ could be used at a dose of 20mg/ L since both provided the best results in terms of hatching quality. Moreover, using chemicals to hatch fairy shrimp cysts in non-aeration conditions presents better results compared to using chemicals with aeration.

INTRODUCTION

Fairy shrimp (*Streptocephalus* sp.) is considered to have great potential as a live food for freshwater aquatic animals. Using fairy shrimp as live food has the following advantages: small larval size, slow-moving, contains essential amino acids, high pigment level, unsaturated fatty acids contain two or more double bonds **(Munuswamy *et al.*, 1997)**, 55% protein content, and19% lipid content **(Velu & Munuswamy, 2007)**. Fairy shrimp is also used as food for some freshwater fish species, such as tilapia *Oreochromis* sp. **(Ali & Dumond, 1995)**, the angelfish *Peterophylum scalare* **(Velu & Munuswamy, 2003)**, sturgeon *Acipenser persicus* **(Namin *et al.*, 2007)**, and the blue gourami *Trichogaster trichopterus* **(Salma *et al.*, 2013)**. The high carotenoid content in fairy shrimp also helps improve growth and pigmentation in both fish and shellfish **(Sriputhorn & Sanoamuang, 2011)**. Moreover, the goldfish *Carassius auratus* fed fairy shrimp recorded growth comparable to that fed with *Artemia* **(Velu & Munuswamy, 2007)**. Therefore, fairy shrimp is a highly suitable alternative to *Artemia* in freshwater aquaculture in Asia.

However, fairy shrimp have a low hatching rate (1.2%), do not have synchronic hatching, and hatching only begins after 7 days of incubation **(Alejos *et al*., 2021)**. Additionally, the hatching rate decreases the longer the eggs are immersed in water after laying **(Seangphan *et al*., 2005)**. **Van Stappen (1996)** and **Robbin *et al*. (2010)** reported that cysts need to be activated from dormancy by many methods, such as sun drying, immersion in high-salinity brine, and using H_2O_2 to achieve a higher hatching rate. Therefore, the use of various concentrations of H_2O_2 and $NaHCO_3$ was tested to eliminate dormancy and induce hatching, and their effects on hatching quality were evaluated to serve as a basis for further research.

MATERIALS AND METHODS

1. Effect of treated substances on fairy shrimp hatching perfomance

Fairy shrimp cysts collected from our previous cultures were used for the present experiment. Cysts were soaked for 1 hour in chloride-free tap water before incubation **(Soorgeloos *et al*., 1980)**. For incubation, 100 fully shaped eggs were siphoned out from a container using a 1-ml pipette and transferred into Petri dishes (diameter: 90cm) containing 10mL of water (35 dishes in total). Then, H_2O_2 (has a concentration of 30% w/w, AR, Xilong scientific company, Guangdong, China) at three different dosages (3, 5, and 7%) and $NaHCO_3$ (activity normaly is ≥99.5%, AR, Xilong scientific company, Guangdong, China) at three different dosages (10, 15, and 20g/ L) were added to the incubation solution. The dishes were then placed in an incubation room at 28±1°C for incubation. The hatching performance of preserved cysts was then compared to the hatching performance of untreated cysts. The experimental setup is shown in Table (1).

Table 1. Experimental setup for fairy shrimp incubation using different substances

Treatment coded	Substance applied	Dosage
Control	Untreated eggs	
H-3%	H_2O_2	3%
H-5%	H_2O_2	5%
H-7%	H_2O_2	7%
Na-10g	$NaHCO_3$	10g/ L
Na-15g	$NaHCO_3$	15g/ L
Na-20g	$NaHCO_3$	20g/ L

The experimental setup corresponded to seven treatments, with five replicates (i.e., five Petri dishes) for each treatment.

2. Effect of incubation condition on fairy shrimp hatching performance

The fairy shrimp samples were incubated in Falcon tubes with 2000 cysts/30 mL of water and 3% H_2O_2 or 20g/ L of $NaHCO_3$ added to the incubation tubes, corresponding to designed treatments. Aeration and non-aeration treatment conditions

were applied to treated and untreated cysts during incubation. This corresponded to six treatments, and each treatment was repeated three times. Cysts were incubated at 28±1℃.

Table 2. Experimental setup for fairy shrimp cysts incubation under different conditions

Treatment code	Substance added	Aeration applied
C-non	Untreated eggs	No
C-Aer	Untreated eggs	Yes
H-non	H_2O_2	No
H-Aer	H_2O_2	Yes
Na-non	$NaHCO_3$	No
Na-Aer	$NaHCO_3$	Yes

Data collection and analysis

Observations of the hatching performance of fairy shrimp cysts at different time intervals (e.g., 12 to 168 hours) included hatchability percentage, hatching beginning and hatching period.

Hatching percentage was calculated as follows: total number of *nauplii* hatched/total number of eggs incubated. Monitoring the hatching rate every 2 hours from the appearance of the first nauplius until the end of the experiment (the last nauplius to hatch) was obtained by counting the number of nauplii that appeared at every 2 hours of incubation.

Hatching beginning is the time when the first nauplius appeared during incubation, while hatching period is the time for the last nauplius hatched.

Statistical analysis

Data were processed with Excel software and using Statistica 7.0 software with one-way analysis of variance (ANOVA) method to compare errors. Significant differences between treatments at $P<0.05$ were detected using Tukey's test.

RESULTS AND DISCUSSION

1. Effect of treated substances on fairy shrimp hatching perfomance

The effects of using different H_2O_2 concentrations on the hatching rate of fairy shrimp are presented in Table (1). The results indicate that the hatching rate did not increase significantly ($P\geq0.05$) when adding H_2O_2, except for the treatment supplemented with H_2O_2 at a dose of 3%, which was significantly higher ($P<0.05$) than the control. **Alejos *et al.* (2021)** reported that fairy shrimp cysts from Thailand have a very low hatching rate. When incubated at 32°C, the hatching rate was only 1.47%, and the incubation time was prolonged to 8 days. In the current experiment, when hatched at room temperature (30℃), the hatching rate reached 4%. However, when 3% H_2O_2 was

added, the hatching rate increased threefold compared to untreated cysts (the control). However, in the H-5% and H-7% treatments, the hatching rate increased very minimally when compared to the control, by 1.8 times and 1.1 times, respectively. According to **Robbins *et al*. (2010)**, H_2O_2 is an oxidizer, has strong oxidizing properties, and is thus a strong bleaching agent used as a disinfectant. Regarding oxidants at higher concentrations, with the stronger oxidizing ability of H_2O_2, soaking time can also have an abrasive effect on cyst shells. The hatching time for all chemically-treated cysts was 2 to 8 hours later than in the control. Some previuos studies reported that H_2O_2 addition improved the hatching rate of *Artemia* cysts **(Van Stappen, 1996; Van Stappen *et al*., 1998; Kryakhova *et al*., 2023)**.

Table 3. Effect of substances on hatching characteristics. Different supperscripts in the same column denote significant differences (P<0.05)

Treatment coded	Hatching percentage (%)	Hatching beginning (h)	Hatching Period (h)
Control	4,0±1,0^{a}	18,34±0,00^{a}	168,00±0,00^{b}
H-3%	12,0±1,7^{b}	24,36±3,29^{b}	121,40±3,29^{a}
H-5%	6,7±0,6^{a}	26,10±3,63^{c}	120,80±3,63^{a}
H-7%	4,7±1,2^{a}	26,40±4,56^{c}	120,40±4,56^{a}
Na-10g	13,7±0,09^{b}	21,40±1,41^{b}	120,00±1,41^{b}
Na-15g	19,3±0,02^{c}	23,37±1,10^{c}	120,40±4,77^{b}
Na-20g	20,4±0,06^{c}	24,50±1,79^{d}	119,00±6,48^{b}

The various concentrations of $NaHCO_3$ improved the hatching rates of fairy shrimp, with the hatching rate ranging from 13.7 to 20.4% (Table 3), which is significantly higher than the control. The concentrations of $NaHCO_3$ added during the incubation of fairy shrimp cysts are proportional to the hatching rate, increasing by 3 to 4.4 times when compared to the control.

The experimental results indicate that using $NaHCO_3$ to improve the hatching rate of fairy shrimp is better than using H_2O_2. $NaHCO_3$ is a weak acid salt. Thus, when used at high doses, this chemical will slowly corrode and soften the shells of fairy shrimp without damaging the embryo—like H_2O_2—resulting in a better hatching rate. Based on the above results, $NaHCO_3$ can be used as a hatching rate stimulant for fairy shrimp.

The results showing the start and hatching period of fairy shrimp (Table 3) indicate that the untreated cysts had an early hatching time, which occurred approximately 18 hours after incubation. The first nauplius appeared in the treated cysts,

The hatching beginning lasted from 24.36 to 26.40 hours, including eggs using H_2O_2 at concentrations of 3, 5, and 7%

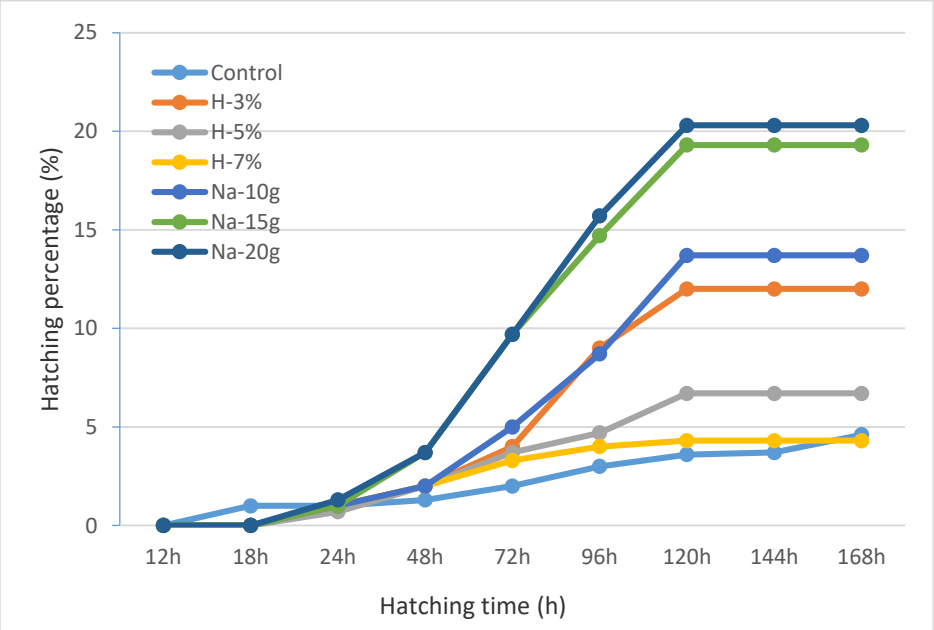

Fig. 1. Hatching rates of fairy shrimp cysts

Although the time it took for eggs to start hatching in treatments using H_2O_2 or $NaHCO_3$ was longer than that not using it, the total hatching time was greatly reduced. Fairy shrimp eggs have a total hatching period of 168 hours; however, when using H_2O_2 or $NaHCO_3$, this was reduced to 120 hours.

The aforementioned results reveal that using H_2O_2 has a great impact on improving the hatching rate for fairy shrimp, especially at low doses (3%), which produce the best results. Additionally, the interaction between chemical dosage has a great influence on the hatching rate of fairy shrimp eggs. H_2O_2 also has an impact on the hatching beggining and hatching period of fairy shrimp cysts. Although the time to start hatching cysts in treatments using H_2O_2 was longer than in the control treatments, the hatching period was shorter. This result is similar to that in the present experiment. Furthermore, when compared to *Artemia franciscana* cysts, fairy shrimp eggs have a rather slow start time and a long hatching period (from 120–168 hours), while *A. franciscana* has a total hatching time ranging from 27-48 hours **(Saygi, 2003)**.

The results of fairy shrimp cysts hatching at the beginning and during the hatching period (Table 3) indicate that the group of eggs that started hatching early (approximately 18 hours after incubation) showed nauplii at the same time as the control group. The egg group had a hatching time lasting from 21.40 to 24.50 hours, including groups of eggs using $NaHCO_3$ with concentrations of 10, 15, and 20g/ L.

In the control treatment, the eggs began to hatch much earlier than in the treatments using $NaHCO_3$ (18 hours compared to 21.40–24.50 hours). Although the time it takes for eggs to start hatching when using $NaHCO_3$ is longer than when not using it, the total hatching time is greatly shortened. Fairy shrimp cysts hatching period decreased the most (about 48 hours) (Table 3). Similar to using H_2O_2, when using $NaHCO_3$ to improve the hatching rate of fairy shrimp eggs, the eggs required a longer period of time to begin hatching. The water pH level increased when $NaHCO_3$ was added to incubated water, however this increase in the pH level negatively affects the hatching rate of crustacean eggs **(Kerul & Talarczyk, 2023)**. Additionally, $NaHCO_3$ is a weakly corrosive chemical. Thus, when it is exposed to egg shells, $NaHCO_3$ will slowly erode them so that the eggs have a longer time to start hatching. As such, the hatching rate increases when the $NaHCO_3$ dosage increases.

The results from Fig. (1) also show that when compared to hydrogen peroxide, using $NaHCO_3$ is more effective for increasing the hatching rate.

2. Effects of aeration on certain hatching quality indicators for fairy shrimp eggs

Aeration affects the hatching rate of fairy shrimp eggs. The results for hatching quality are presented in Table (4), showing that hatching without aeration produced a higher hatching rate than with aeration.

The hatching rate of fairy shrimp eggs ranges from 2.6 to 20.1%. Here, the lowest hatching rate of 2.6% was recorded in the control treatment with aeration, while the highest hatching rate of 20.1% was recorded in the $NaHCO_3$-treated cysts with no aeration applied.

The experimental results show that using chemicals without aeration to improve the hatching rate of fairy shrimp eggs was better than when using chemicals with aeration.

The results of the hatching beginning and total hatching time for fairy shrimp eggs (Table 4) indicate that the non-treated cysts started hatching early (approximately 18 hours after incubation), while treated cysts had a hatching time lasting from 21.40 to 24.50 hours, including groups of eggs using $NaHCO_3$ at a concentration of 20g/ L and cysts using H_2O_2 at a concentration of 3% under both aeration and aeration-free conditions.

Table 4. Effects of supplying aeration on the hatching performance of fairy shrimp eggs. Different supperscripts in the same column denote significant differences ($P<0.05$)

Treatment coded	Hatching rate (%)	Hatching beginning (h)	Total hatching time (h)
C-non	4,6±0,1^{a}	18,00±0,00^{a}	168,00±0,00^{d}
C-Aer	2,6±0,1^{a}	18,10±0,00^{a}	168,00±0,00^{d}
H-non	12,3±0,1^{c}	24,36±1,41^{b}	120,00±1,41^{bc}
H-Aer	8,5±0,07^{b}	24,56±1,41^{b}	120,00±1,41^{bc}
Na-non	20,1±0,02^{e}	24,50±1,10^{c}	120,40±4,77^{a}
Na-Aer	16,4±0,06^{d}	25,00±1,79^{c}	119,00±6,48^{a}

CONCLUSION

The hatching rate of fairy shrimp is greatly increased by higher H_2O_2 and $NaHCO_3$ concentrations in incubation water.

The addition of 3% H_2O_2 can improve fairy shrimp egg hatchability with the addition of $NaHCO_3$ at a dose of 20mg/ L.

The aeration-free incubation condition for fairy shrimp eggs produced a higher hatching rate when compared to the aeration condition.

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