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Effect of temperature on the hatching of fairy shrimp *Branchinella thailandensis* from Thailand

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Abstract

The effect of temperature on the hatching of fairy shrimp *Branchinella thailandensis* from Thailand was studied. The cysts were kept at different temperatures regimes [ambient (26-29ºC), 30°C, 32°C, and 34°C, and the effect of the resulting experimental condition on hatching was examined until no hatching was observed. Results indicated that temperature affects the hatching of the Thai fairy shrimp *B. thailandensis* cysts. The hatching of the fairy shrimp could take place once at 34ºC and can be stimulated at 32ºC but fluctuating temperature (26-29ºC) and 30ºC may reduce hatching success. However, the incubation period took longer, and hatching success was extremely low which suspected a consequence of improper production technique and processing of cysts used in this study. In conclusion, hatching of fairy shrimp *B. thailandensis* can be improved at 34ºC but optimal at 32ºC.

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Introduction

Fairy shrimp are one of the most unique crustacean groups that have adopted their life history, including early hatching, rapid maturation, and early start of egg production, to effectively inhabit temporary ponds but little is known regarding the environmental conditions present in temporary ponds that may stimulate hatching of resting eggs (Dararat *et al.*, 2011). Several environmental variables have been suggested as hatching cues for temporary pool invertebrates, such as water level (Hall, 1959), conductivity (Sam and Krishnaswamy, 1979; Brendonck *et al.*, 1998), oxygen concentration (Moore, 1967), light regime (Mitchell, 1990), and temperature (Brendonck *et al*, 1998; Brendonck and Riddoch, 2001).

In Thailand, three species of fairy shrimps have been discovered (Dararat *et al*, 2011) and because of the continuous availability of large numbers of cysts and its economic importance in aquaculture, efforts have been focused on the cultivation of these new species to use them as a new live food for freshwater aquatic animals such as prawns, shrimp, and ornamental fish Saengphan *et al.*, 2005; Saengphan *et al*, 2006; Sanoamuang and Saengphan, 2006; Boonmaket al., 2007; Saengphan and Sanoamuang, 2009; Sriputhorn and Sanoamuang, 2011). However, a major constraint in the use of freshwater branchiopods like the fairy shrimps in aquaculture and aquariology is the asynchronous, unpredictable, and often population-dependent nature of hatching of their cysts (Brendonck *et al*, 1996). These are also a major drawback to the use of cysts-based tests in aquatic toxicology since enough numbers of test organisms of uniform age are required at a predictable time after incubation of the cysts (Centeno *et al*, 1993). In most countries wherein fairy shrimp are not naturally thriving and no existing cysts production, researchers and aquaculturists usually rely on to purchase online which entails much higher cost, thus, the hatching rate is very important to recover the cost.

Limited studies have been conducted to determine the hatching of Thai fairy shrimps. Saengphan *et al* (2005) investigated the cyst hatching pattern of the Thai Fairy Shrimp *B. thailandensis* concerning brood order, and responses of cysts to wet and dry periods. In this experiment, cysts of *B. thailandensis* were incubated at room temperature ranging from 21-34ºC for hatching. Recently, Dararat *et al* (2011) differentiated the daily hatching success at 27ºC of the three species of Thai fairy shrimps, *Streptocephalus sirindhornae*, *S. siamensis*, and *B. thailandensis*.

The hatching of activated cysts of large freshwater branchiopods (Crustacea: Branchiopoda: Anostraca, Notostraca, Conchostraca) with few exceptions, are affected by specific conditions that may even differ among conspecific populations, which in general, each species (or even population) has a specific temperature range or regime for optimal hatching performance (Brendonck, 1996) which is still unknown for the Thai fairy shrimp *B. thailandensis*. Thus, this study aims to determine the effect of temperature on the hatching of fairy shrimp *B. thailandensis* from Thailand.

Materials and methods

Study site

The study was conducted at the National Institute of Molecular Biology and Biotechnology (NIMBB), University of the Philippines Visayas (UPV), Miagao, Iloilo, Philippines.

Source and preparation of cysts

Thai fairy shrimp *B. Thailandensis* cysts were purchased online via eBay and it took 17 days the item to arrive. The cysts were stored at room temperature before the experiment. To remove impurities, cysts were first passed through a 500µm to 250µm sieve (U.S.A standard testing sieved). A sample of 0.75g cysts were weighed in electronic balance (Ohaus™ PR Series) and diluted several times in a beaker. The cysts were manually taken from the diluted sample using 250µL micropipette (Thermo Scientific F1-ClipTip Fixed Volume), placed them in Sedgewick Rafter Counting Chambers (Graticules S52), and counted under stereo zoom microscope (SMZ161 Series–Motic).

Experiment proper

The effect of temperature on the hatching of Thai fairy shrimp *B. thailandensis* was evaluated indoor using 12 units transparent plastic hatching container (1L capacity) with conical bottom containing 500mL distilled water (Maeda-martinez *et al*, 1995) installed with airlines in the bottom to provide constant aeration and kept the cysts in continuous suspension. The containers were sub-dividedly placed in 4 units thermostatically maintained (Aqua Zonic® 100w heater) hatching incubator (65L capacity) and covered with clear plastic cellophane to limit the intrusion of pests especially during nighttime. Two units' daylight fluorescent lamps (FSV1/40, FS40/T10D) were installed 3m above the hatching containers to provide continuous illumination as suggested by Mitchell (1990). Since the set-up is an open type, it is affected by natural light during the daytime. Four temperature treatments with three replicates were tested [26-29ºC (ambient), 30ºC, 32ºC, and 34ºC]. Each hatching incubator containing 3 units of hatching container represents 1 temperature treatment. The fairy shrimp cysts were stocked at 0.5g L^{-1}. Water parameters were constantly monitored and kept at the desired temperature in each treatment, pH 9 ± 0.5, and DO 6.5mg L^{-1}. Change water with the same temperature in each treatment was done on the 5th, 8th, and 10th day of the experiment using bottled distilled water floated in each incubator. Hatching was closely monitored until no hatching was observed.

During harvesting, lights and heaters were turned off before the hatching containers were removed from the hatching incubators. The nauplii and the cysts (unhatched and empty shells) were removed from each container by draining using the air host, placed them in a conical transparent plastic container shaded with black cloth on the upper part focusing a light source on the bottom part, and allowed them to settle for around 30 minutes. The nauplii were collected first on a filter using a fine mesh scoop (100µm). Since few hatchings were observed on the eight-day, all cysts (unhatched and empty shells) were collected, washed with topwater, and re-stocked for hatching. The hatching containers were cleaned, installed back in the thermostatically maintained hatching incubators, and filled with new distilled water before re-stocking of the cysts.

During counting, concentrated fairy shrimp nauplii were poured in a 500mL beaker provided with gentle aeration to uniformly mixed the organisms. A 50 mL sub-sample was taken using a 3mL transfer plastic pipette and poured in a 300-500mL beaker depending on the concentration of the nauplii. The nauplii were then manually taken from the sample using 1000µL micropipette (Thermo Scientific F1-ClipTip Fixed Volume), placed them in Sedgewick Rafter Counting Chambers (Graticules S52), and counted under stereo zoom microscope (SMZ161 Series – Motic).

Determination of hatching percentage was made with the formula

$H (\%) = NH/CSD \times 100$

where:

H (%) = Hatching Percentage

NH = Nauplii hatching

CSD = Cysts stocking density

Statistical analysis

After the experiment, results were analyzed using the Statistical Package for Social Sciences (SPSS) version 20 software. Data were tested for homogeneity of variance using Levene's test. The one-way analysis of variance (ANOVA) was used on data that passed the tests while those that did not were subjected to log or arcsine transformation. When a significant difference between treatment means confirmed, Tukey's test was applied, with a significance level of $p \le 0.05$. For the data that does not have a normal distribution, the nonparametric test was performed using the Kruskal-Wallis test. Pairwise comparisons were applied when a significant difference was confirmed at the significance level of $p \le 0.05$.

Results and discussion

The hatching of Thai fairy shrimp *B. thailandensis* cysts at different temperature regimes are presented in fig. 1. No hatching of the incubated cysts existed from day 1 to 6 at all temperature regimes.

Hatching begins on the 7th day (1600H) at lower temperatures (ambient, 30ºC & 32ºC) and 8th day (0500H) at a higher temperature (34ºC). However, only very few hatchings were observed at lower temperatures (ambient, 30ºC & 32ºC) on the 7th day which eventually increased on the following day unlike at the highest temperature (34ºC) wherein cysts hatching occurred only once on the 8th day. A highly significant difference ($p < 0.01$) was observed on the number of counted nauplii and hatching percentage of the incubated cysts at different temperatures regimes on the 8th day including the cumulative results with higher counting and hatching at 32ºC. On the other hand, hatching of the re-incubated cysts was only observed at ambient temperature (26-29ºC) on the 10th day and no succeeding hatching was observed afterward at all temperature regimes.

The importance of temperature for anostracan egg hatching has been demonstrated by several researchers (Belk and Cole, 1975; Horne, 1967; Hathaway and Simovich, 1996). In this study, results indicated that temperature affects the hatching of the Thai fairy shrimp *B. thailandensis*. This agrees with several studies wherein the hatching of fairy shrimp species has been affected when incubated at different temperature regimes ((Al-Tikrit and Grainger, 1990; Brendonck *et al*., 1996; Beladjal *et al*, 2003; Atashbar *et al*., 2012). The hatching of *B. thailandensis* could take place once at 34ºC and can be stimulated at 32ºC. Other studies reported the highest cumulative hatching success for *Streptocephalus torvicornis* at 24°C (Atashbar *et al*., 2012) and *Phallocryptus spinosa* at 22°C (Hulsmans *et al*., 2006). In some species of fairy shrimps, however, there is no optimal temperature range for hatching but an optimal regime of successive temperature conditions depending on the developmental stage (Brendonck, 1996). For instance, the hatching of *Streptocephalus seali* (Moore, 1967) could be further stimulated by fluctuating water temperatures within the optimal temperature range. In *S. seali*, fluctuating temperatures within 19-23ºC increased hatching in comparison with incubation at a constant temperature within this range (Moore, 1967). In the study of Al-Tikrit and Grainger (1990), some indications that changing temperature may stimulate the hatching of *Tanymastix stagnalis* resting eggs. In contrast, fluctuating temperature (26-29ºC) and at 30ºC can reduce the hatching of *B. thailandensis* in this study. Summarizing the results obtained by several authors shows that optimal hatching in different species of fairy shrimps is species-specific. In this study, however, the incubation period took longer, and hatching success is extremely low. The lowest cumulative hatching percentage was obtained at 30ºC (0.08%) followed by ambient (0.37%), 34ºC (0.61%) with highest hatching at 32ºC (1.47%). Saengphan *et al* (2005) reported a 0-99.33% hatching percentage of *B. thailandensis* where cyst hatching mostly took place within 24 hours after incubation at room temperature (21-34ºC) using deposited cysts of the first, sixth, and eleventh broods of the species under wet and dry conditions. In this experiment, freshly laid cysts both wet and dry conditions which were immediately incubated did not hatch while the undried cysts immersed in their parental medium for 4 weeks showed the highest hatching in all brood treatments (76.67, 94.67, and 99.33% of the first, sixth, and eleventh broods, respectively).

This shows that the cysts require a period of retention in the parental medium for 2-4 weeks to complete their embryonic development before hatching and drying is not essential for cyst hatching of *B. thailandensis*. In the study of Dararat *et al* (2011), *B. thailandensis* obtained the highest hatchability of 87.67ºC and shortest hatching time within 3 days at 27ºC compared with other two species of Thai fairy shrimps *S. sirindhornae* and *S. siamensis* following the egg preparing methods described by Saengphan *et al* (2005).

Fig. 1. *B. thailandensis* cysts from Thailand.

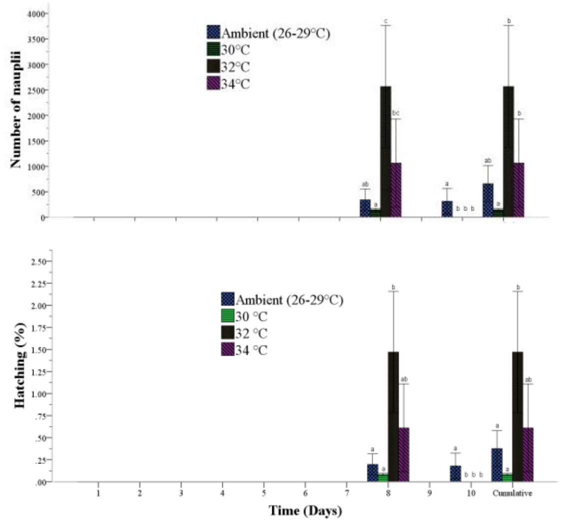

Fig. 2. Hatching of Thai fairy shrimp *B. thailandensis* cysts at different temperatures regimes. (A) the number of nauplii hatched; (B) hatching percentage. Data are expressed as mean ± SE. *Equal letters do not show significant differences in treatments by the Tukey's test (*p*<.05) (Day 8 and Cumulative) and Pairwise comparisons (*p*<.05) (Day 10).

The results of the experiments (Saengphan, Shiel & Sanoamuang, 2005; Dararat, Starkweather & Sanoamuang, 2011) raises the question of the quality of the cysts purchased from Thailand (Fig. 1). According to Van Stappen (1996), the hatching capacity of the full cysts depends on the number of factors. First is the degree of diapause termination wherein cysts that are still in diapause do not hatch, even under favorable hatching conditions. Second is the energy content of cysts which may be too low to build up enough levels of glycerol to enable breaking and hatching, and third is the amount of dead or non-viable, abortic embryos. The second and third factors are a consequence of, for example, improper processing and or storage. Although the embryonic development of *B. thailandensis* seemed to continue at a slower rate during drying, however, most of the embryos might continue or have stopped developing or have died in the dry (Saengphan *et al.*, 2005). In line with this, it is suspected that most of the cysts used in this study did not complete their embryonic development which has been adversely affected by a mechanism leading to a much lower hatching success which may be a consequence of improper production technique and processing of cysts. This agrees with the low hatching percentage of *B. thailandensis* (2.67 and 0.67% of the sixth and eleventh broods, respectively) which probably represents diapausing cysts (Saengphan *et al.*, 2005).

Conclusion

The hatching of fairy shrimp *B. thailandensis* can be improved at 34°C but optimal at 32°C. Although the common temperatures usually fairy shrimps occur during the rainy season in Thailand ranges from 24-26°C (Atashbar *et al.*, 2012), fluctuating temperatures from 26-29°C and at 30°C constant temperature may reduce the hatching of *B. thailandensis*. The viability of encysted embryos produced by Anostracans in the sediments for years (Marcus, 1996) and drought-resistant cysts produced by large branchiopods (Longhurst, 1955) may be related to higher hatching success of *B. thailandensis* at a higher temperature (32-34°C).

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