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Original Research Article

Effect of Temperature on the Hatching of Fairy Shrimp *Branchinella thailandensis* Sanoamuang, Saengphan & Murugan, 2002 (Branchiopoda: Anostraca) from Thailand

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ABSTRACT:
The hatching of fairy shrimp *Branchinella thailandensis* from Thailand was studied. The fairy shrimp cysts were subjected to different temperature regimes [ambient (26-29^{o}C), 30°C, 32°C, and 34°C] and examined the experimental condition's effect on hatching until no hatching occurred. Results indicated that temperature affects the hatching of the Thai fairy shrimp *B. thailandensis* cysts. The hatching of the fairy shrimp could occur once at 34^{o}C and can be stimulated at 32^{o}C, but fluctuating temperatures (26-29^{o}C) and 30^{o}C may reduce hatching success. However, the incubation period took longer, and hatching success was extremely low, suspected of improper cysts production technique and processing. In conclusion, the hatching of fairy shrimp *B. thailandensis* can be improved at 34^{o}C but optimal at 32^{o}C.

Keywords: *Anostraca, B. thailandensis, Cysts, Temperature, Nauplii, Hatching, Cumulative*

INTRODUCTION

Fairy shrimp are unique crustacean groups that have adopted their life history, including early hatching, rapid maturation, and early start of egg production, to inhabit temporary ponds effectively. However, little is known

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regarding the environmental conditions present in temporary ponds that may stimulate the hatching of resting eggs present in temporary ponds (Dararat, Starkweather, & Sanoamuang, 2011). Several environmental variables have been suggested as hatching cues for temporary pool invertebrates, such as water level (Hall, 1959), conductivity (Sam & Krishnaswamy, 1979; Brendonck, Riddoch, Van de Weghe, & Van Dooren, 1998), oxygen concentration (Moore, 1967), light regime (Mitchell, 1990), and temperature (Brendonck et al., 1998; Brendonck & Riddoch, 2002).

In Thailand, three species of fairy shrimps have been discovered (Dararat et al., 2011). Because of the continuous availability of large numbers of cysts and their economic importance in aquaculture, efforts have been focused on the cultivation of these new species to use them as fresh live food for freshwater aquatic animals such as prawns, shrimp, and ornamental fish (Saengphan, Sriputhorn, & Sanoamuang, 2006; Boonmak, Saengphan, & Sanoamuang, 2007; Plodsomboon & Sanoamuang, 2007; Saengphan & Sanoamuang, 2009; Sriputhorn & Sanoamuang, 2011). However, a significant constraint in using freshwater branchiopods like the fairy shrimps in aquaculture and aquariology is the asynchronous, unpredictable, and often population-dependent nature of hatching cysts (Brendonck, Centeno, & Persoone, 1996). According to Centeno, Brendonck, and Persoone (1993), these are also significant drawbacks to using cysts-based tests in aquatic toxicology since enough test organisms of uniform age are required at a predictable time after incubating the cysts. On the other hand, in most countries wherein fairy shrimp are not naturally thriving and no cysts production, researchers and aquaculturists usually rely on purchasing online, which entails a much higher cost. Thus, the hatching rate is critical to recover the cost.

Limited experiments have been conducted to determine the hatching of Thai fairy shrimps. Saengphan, Shiel, and Sanoamuang (2005) investigated the cyst hatching pattern of the Thai Fairy Shrimp *B. thailandensis* concerning brood order and cyst's responses to wet and dry periods. In this experiment, cysts of *B. thailandensis* were incubated at room temperature ranging from 21-34^{o}C for hatching. Recently, Dararat et al. (2011) differentiated the daily hatching success at 27^{o}C of the three Thai fairy shrimps species, *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. In general, each species (or even population) has a particular 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

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

Thai fairy shrimp *B. Thailandensis* cysts were purchased online via eBay, and it took 17 days for the item to arrive. The cysts were stored at room temperature before the experiment. Cysts were first passed through a 500µm to 250µm sieve (U.S.A standard testing sieved) to remove cyst's impurities. A sample of 0.75g cysts was 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 in Sedgewick Rafter Counting Chambers (Graticules S52), and counted under stereo zoom microscope (SMZ161 Series – Motic).

The effect of temperature regimes 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, Belk, Obregon-Barboza,

& Dumont, 1995). Airlines were installed in the bottom of the containers 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 daylight fluorescent lamps (FSV1/40, FS40/T10D) were installed 3m above the hatching containers to provide continuous illumination (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 [26-29°C (ambient), 30°C, 32°C, and 34°C]. Each hatching incubator containing three units of hatching container represents one 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 ± 0.5, and DO 6.5 mg 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. We closely monitored the hatching of the fairy shrimp until no hatching was observed.

During harvesting, lights and heaters were switched 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 hose, placed 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 (100 µm). Since there were few hatchings on the eight-day, all cysts (unhatched and empty shells) were collected, washed with tap water, 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 the cysts.

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

Determination of hatching percentage was calculated with the formula
H (%) = NH/CSD × 100
where:
H (%) = Hatching Percentage
NH = Nauplii hatching
CSD = Cysts stocking density

Results were analyzed using the Statistical Package for Social Sciences (SPSS) version 20 software. Using Levene's test, data were tested for homogeneity of variance. Data that passed the tests were analyzed using the one-way analysis of variance (ANOVA), while those that did not were subjected to log or arcsine transformation. When a significant difference between treatment means was confirmed, Tukey's test was applied, with a significance level of $p \leq 0.01$. The nonparametric test was performed using the Kruskal-Wallis test for the data that does not have a normal distribution. Pairwise comparisons were applied when a significant difference was confirmed at the significance level of $p \leq 0.01$.

RESULTS AND DISCUSSION

The hatching of Thai fairy shrimp *B. thailandensis* cysts at different temperature regimes are presented in Table 1 and 2. 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, 30°C & 32°C) and the 8th day (0500H) at a higher temperature (34°C). However, only very few hatchings were observed at lower temperatures (ambient, 30°C & 32°C) on the 7th day, which eventually increased on the following day, unlike at the highest temperature (34°C), cysts hatching occurred only once on the 8th day. A highly significant difference ($p < 0.01$) was observed in the number of counted nauplii and hatching percentage of the incubated cysts at different temperature regimes on the 8th day, including the cumulative results with a higher number and hatching at 32°C. On the other hand, hatching of the re-incubated cysts was only observed at ambient temperature (26-29°C) on the 10th day, and no succeeding hatching was observed afterward at all temperature regimes.

Table 1: Number of Thai fairy shrimp *B. thailandensis* cysts hatched at different temperature regimes

Temperature	Day		Cumulative
	8	10	
Ambient (26-29°C)	344.00±107.62[ab]	316.33±124.31[a]	660.33±177.90[ab]
30°C	148.00±12.00[a]	0.00±0.00[b]	148.00±12.00[a]
32°C	2566±599.34[c]	0.00±0.00[b]	2566±599.34[b]
34°C	1064±430.75[bc]	0.00±0.00[b]	1064±430.75[b]

Data are expressed as mean ± SE. *Equal letters do not show significant differences in treatments by the Tukey's test ($p < 0.01$) (Day 8 and Cumulative) and Pairwise comparisons ($p < 0.01$) (Day 10).

Table 2: Hatching percentage of Thai fairy shrimp *B. thailandensis* cysts at different temperature regimes

Temperature	Day		Cumulative
	8	10	
Ambient (26-29°C)	0.19±0.06[a]	0.18±0.06[a]	0.37±0.10[a]
30°C	0.08±0.00[a]	0.00±0.00[b]	0.08±0.00[a]
32°C	1.47±0.34[b]	0.00±0.00[b]	1.47±0.34[b]
34°C	0.61±0.24[ab]	0.00±0.00[b]	0.61±0.24[ab]

Data are expressed as mean ± SE. *Equal letters do not show significant differences in treatments by the Tukey's test ($p < 0.01$) (Day 8 and Cumulative) and Pairwise comparisons ($p < 0.01$) (Day 10).

Figure 1: *B. thailandensis* cysts from Thailand

The importance of temperature for anostracan egg hatching has been demonstrated by several researchers (Belk & Cole,1975; Horne, 1967; Hathaway & Simovich, 1996). In this study, results indicated that temperature affects the hatching of the Thai fairy shrimp *B. thailandensis*, which agrees with several studies wherein the hatching of fairy shrimp species has been affected when incubated at different temperatures regime (Al-Tikrity & Grainger, 1990; Brendonck et al., 1996; Beladjal, Khattabi & Mertens, 2003; Hulsmans et al., 2006; Atashbar et al., 2012). The hatching of *B. thailandensis* could take place once at 34°C and can be stimulated at 32°C. Other studies reported the highest cumulative hatching success for *S. torvicornis* at 24°C and *Phallocryptus spinosa* at 22°C (Hulsmans et al., 2006). However, there is no optimal temperature range for hatching in some fairy shrimps species but an optimal regime of successive temperature conditions based on the developmental stages (Brendonck, 1996). For instance, the hatching of *S. seali* (Moore, 1967) could be further stimulated by fluctuating water temperatures within the optimal temperature range. In *S. seali*, fluctuating temperatures within 19-23°C increased hatching compared to incubation at a constant temperature within this range (Moore,1967). Studies by Al-Tikrit and Grainger (1990) suggest that changing temperature may stimulate the hatching of *Tanymastix stagnalis* resting eggs. In contrast to this observation, our study showed that fluctuating temperature (26-29°C) and at 30°C could reduce the hatching of *B. thailandensis*, suggesting that optimal hatching temperature in different species of fairy shrimps is species-specific.

In this study, however, the incubation period took longer, and hatching success is meager. The lowest cumulative hatching percentage was obtained at 30°C (0.08%) followed by ambient (0.37%), 34°C (0.61%) with highest hatching at 32°C (1.47%). Saengphan et al. (2005) reported a 0-99.33% hatching of *B. thailandensis* where cyst hatching mostly took place within 24 hours after incubation at room temperature (21-34°C) using deposited cysts of the first, sixth, and eleventh broods of the species under wet and dry conditions. However, it was found that freshly laid cysts, whether in wet and dry conditions, did not hatch, even when they were incubated, immediately. The undried cysts immersed in their parental medium for four weeks resulted in the highest hatching in all brood treatments (first, 76.67%; sixth, 94.67%; eleven, 99.33%). The study shows that the cysts require retention in the parental medium for 2-4 weeks to complete their embryonic development before hatching. 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 87.67% and shortest hatching time within three days at 27°C compared with the other two species of Thai fairy shrimps, *S. sirindhornae* and *S. siamensis*, following the egg preparing methods described by Saengphan et al. (2005).

The results of the above experiments (Saengphan et al., 2005; Dararat et al., 2011) raise the quality of the cysts purchased from Thailand (Figure 1). According to Van Stappen (1996), the full cysts hatching capacity depends on several factors. First is the degree of diapause termination, where cysts that are still in diapause do not hatch, even under favorable hatching conditions. The second is the cyst's energy content, which may be too low to build up enough glycerol levels to enable breaking and hatching, and the 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, most of the embryos might continue or have stopped developing or have died in the dry condition (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 much lower hatching success and a consequence of improper cysts production techniques, processing, and storage. The results agree with the low hatching percentage of *B. thailandensis* (2.67 and eleventh, 0.67% of the sixth and eleventh broods, respectively), representing diapausing cysts (Saengphan et al., 2005). On the other hand, Thaimuangphol & Sanoamuang (2017) demonstrated that the optimal egg viability storage conditions for two commercial fairy shrimps are at- 18°C for at least 24 months with high hatchability in *B. thailandensis* (80%) and *S. sirindhornae* (60%) under dry, dark, anoxic conditions.

CONCLUSION

The hatching of fairy shrimp *B. thailandensis* can be improved at 34ºC but optimal at 32ºC. Although the typical temperatures usually fairy shrimps occur during the rainy season in Thailand ranges from 24-26ºC (Atashbar et al., 2012), fluctuating temperatures from 26-29ºC and at 30ºC 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 the higher hatching success of *B. thailandensis* at a higher temperature (32-34ºC).

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Conflicts of Interest: None at all

REFERENCES

1. Dararat, W., Starkweather, P. L., and Sanoamuang, L. O. (2011). Life history of three fairy shrimps (Branchiopoda: Anostraca) from Thailand. *Journal of Crustacean Biology*, 31(4), 623–629. https://doi.org/10.1651/10-3410.1
2. Hall, R. E. (1959). The development of eggs of *Chirocephalus diaphanus* Prévost in relation to depth of water. *Hydrobiologia*, 14(1), 79–84. https://doi.org/10.1007/BF00160819
3. Sam S. T., and Krishnaswamy, S. (1979). Effect of osmomolarity of the medium upon hatching of undried eggs of *Streptocephalus dichotomus* Baird (Anostraca, Crustacea). *Archiv fur Hydrobiologie*, 86(1), 125–130.
4. Brendonck, L., Riddoch, B. J., Van de Weghe, V., and Van Dooren, T. (1998). The maintenance of egg banks in very short-lived pools - a case study with anostracans (Branchiopoda). *Archiv Fur Hydrobiologie*, 52, 141-161.
5. Moore, W. G. (1967). Factors affecting egg hatching in *Streptocephalus seali* (Branchiopoda, Anostraca). *Proc. Symp. Crustacea, Hernakulam*, 2, 724–735.
6. Mitchell, S. A. (1990). Factors affecting the hatching of *Streptocephalus macrourus* Daday (Crustacea, Eubranchiopoda) eggs. *Hydrobiologia*, 194(1), 13-22. https://doi.org/10.1007/BF00012108
7. Brendonck, L., and Riddoch, B. J. (2002). Hatching characteristics of the fairy shrimp *Branchipodopsis wolfi* in relation to the stochastic nature of its habitat, desert rock pools. In: Williams WD (ed). *Verhandlungen des Internationalen Verein Limnologiel,* 27(7), 3931–3935. https://doi.org/10.1080/03680770.1998.
8. Saengphan, N., Shiel, R. J., and Sanoamuang, L. (2005). The cyst hatching pattern of the Thai fairy shrimp, *Branchinella thailandensis* Sanoamuang, Saengphan and Murugan, 2002 (Anostraca). *Crustaceana*, 78(5), 513-523. https://doi.org/10.1163/1568540057743123
9. Saengphan, N., Sriputhorn, K., and Sanoamuang, L. (2006). Cultures of Fairy Shrimp in Thailand. Klangnanatham Publishers, Khon Kaen, Thailand.
10. Boonmak, P., Saengphan, N., and Sanoamuang, L. (2007). Biology and fecundity of two fairy shrimp, *Streptocephalus sirindhornae* Sanoamuang, Murugan, Weekers and Dumont, and *Branchinella thailandensis* Sanoamuang, Saengphan, and Murugan. *Khon Kaen University Research Journal*, 12 (2), 125-131.
11. Plodsomboon, S., and Sanoamuang, L. (2007). Effect of salinity on the survival of the Thai fairy shrimp's nauplii (*Branchinella thailandensis* Sanoamuang, Saengphan, and Murugan, 2002). *Journal of Scientific Research*, 6, 165-173.
12. Saengphan, N., and Sanoamuang, L. (2009). Effect of food concentrations on growth and survival of the fairy shrimp *Branchinella thailandensis* Sanoamuang, Saengphan, and

Murugan. *Burapha Science Journal,* 14, 19-28.

13. Sriputhorn, K., and Sanoamuang, L. (2011). Fairy shrimp (*Streptocephalus sirindhornae*) as live feed improves growth and carotenoid contents of giant freshwater prawn *Macrobrachium rosenbergii*. *International Journal of Zoological Research*, 7(2), 138-146. http://dx.doi.org/10.3923/ijzr.2011.138.146

14. Brendonck, L., Centeno, M.D., and Persoone, G. (1996). The influence of processing and temperature conditions on hatching of resting eggs of *Streptocephalus proboscideus* (Crustacea: Branchiopoda: Anostraca). *Hydrobiologia*, 320, 99–105. https://doi.org/10.1007/BF00016810

15. Centeno, M. F., Brendonck, L., and Persoone, G. (1993). Acute toxicity tests with *Streptocephalus proboscideus* (Crustacea: Branchiopoda: Anostraca): influence of selected environmental conditions. *Chemosphere*, 27(11), 2213-2224. https://doi.org/10.1016/0045-6535(93)90133-P

16. Brendonck, L. (1996). Diapause, quiescence, hatching requirements: what we can learn from large freshwater branchiopods (Crustacea: Branchiopoda: Anostraca, Notostraca, Conchostraca). *Hydrobiologia,* 320, 85–97. https://doi.org/10.1007

17. Maeda-martinez, A., Belk, D., Obregon-Barboza, H., and Dumont, H. (1995). A contribution to the systematics of the Streptocephalidae (Branchiopoda, Anostraca). *Hydrobiologia*, 298, 203–232.https://doi.org/10.1007/BF00033815

18. Belk, D., and Cole, G. A. (1975). Adaptational biology of desert temporary-pond inhabitants. In N. F. Hadley (ed.), *Environmental physiology of desert organism s*(pp. 207–226). Dowden, Hutchinson and Ross, Inc., Stroudsburg.

19. Horne, F. R. (1967). Effects of physical-chemical factors on the distribution and occurrence of some southeastern Wyoming phyllopods. *Ecology*, 48(3), 472-477. https://doi.org/10.2307/1932682

20. Hathaway, S. A., and Simovich, M. A. (1996). Factors affecting the distribution and co-occurrence of two Southern California anostracans (Branchiopoda), *Branchinecta sandiegonensis* (Fugate, 1993) and *Streptocephalus woottoni* (Eng, Belk, Eriksen, 1990). *Journal of Crustacean Biology*, 16(4), 669-677. https://doi.org/10.1163/193724096X00757

21. Al-Tikrity, M. R., and Grainger, J. N. R. (1990). The effect of temperature and other factors on the hatching of the resting eggs of *Tanymastix stagnalis* (L.) (crustacea, anostraca). *Journal of Thermal Biology*, 15(1), 87–90. https://doi.org/10.1016/03064565(90)90053-K

22. Beladjal, L., Khattabi, E. M., and Mertens, J. (2003). Life history of *Tanymastigites perrieri* (Anostraca) in relation to temperature. *Crustaceana*, 76, 135-147.https://doi.org/10.1163/156854003321824495

23. Hulsmans, A., Bracke, S., Moreau, K., Riddoch, B. J., De Meester, L., and Brendonck, L. (2006). Dormant egg bank characteristics and hatching pattern of the *Phallocryptus spinosa* (Anostraca) population in the Makgadikgadi Pans (Botswana). *Hydrobiologia*, 571(1), 123–132. https://doi.org/10.1007/s10750-006-0233-x

24. Atashbar, B., Agh, N., Beladjal, L., Jalili, R., and Mertens, J. (2012). Effects of temperature on survival, growth, reproductive and life span characteristics of *Branchinecta orientalis* G. O. Sars, 1901 (Branchipoda, Anostraca) from Iran. *Crustaceana,* 85(Part9), 1099–1114. https://doi.org/10.1163/15685403-00003115

25. Van Stappen, G. (1996). Introduction, biology, and ecology of Artemia. In P. Lavens & P. Sorgeloos (Eds.), Manual on the production and use of live food for aquaculture. *FAO Technical Paper*, 361, 79–106, Rome, Italy. http://hdl.handle.net/1854/LU-4245243

26. Marcus, N. H. (1996). Ecological and evolutionary significance of resting eggs in marine copepods: past, present, and future. *Hydrobiologia*, 320(1-3), 141-152. https://doi.org/10.1007/bf00016815

27. Thaimuangphol, W., and Sanoamuang, L. (2017). Optimal egg viability storage conditions in two commercial fairy shrimps (Crustacea: Branchiopoda: Anostraca) from Thailand. *Aquaculture Research*, 2017, 1–12. https://doi.org/10.1111/are.13320

28. Longhurst, A. R. (1955). A review of the Notostraca. *Bulletin of the British Museum (Natural History)*, 3, 1-57. https://doi.org/10.5962/bhl.part.4119

29. Sanoamuang, Lo., Saengphan, N. and Murugan, G. (2002). First record of the family Thamnocephalidae (Crustacea: Anostraca) from Southeast Asia and description of a new species of Branchinella. *Hydrobiologia*, 486, 63–69. https://doi.org/10.1023/A:1021378113530