Vietnam Journal of Agricultural Sciences

ISSN 2588-1299

VJAS 2020; 3(4): 798-805
https://doi.org/10.31817/vjas.2020.3.4.03

Effects of Temperature on Population Growth and Resting Egg Production of Freshwater Rotifer (*Brachionus calyciflorus*)

Doan Thi Nhinh, Pham Thi Lam Hong, Truong Dinh Hoai & Kim Van Van

Faculty of Fishery, Vietnam National University of Agriculture, Hanoi 131000, Vietnam

Abstract

Resting egg production of rotifers provides critical advantages in larviculture of most fish species due to the reduction in the costs of labour and algae production. The study was conducted to investigate the effects of temperature on population growth and resting egg production of a freshwater rotifer, *Brachionus calyciflorus*, collected in Northern Vietnam. The rotifer was pre-cultured at 27ºC before being transferred to the cultures at 15 and 35ºC while the control was maintained at 27ºC. One-liter beakers filled with 500mL culture medium were used with three replicates for each temperature group. Stock rotifers were inoculated at an initial density of 200 ind ml^{-1} and fed with concentrated fresh algae. The results indicated that population growth rate (r) of rotifers cultured at 27 and 35ºC were significantly higher than that of rotifers at 15ºC while the highest density was attained from the treatment of 27ºC, at 608.3 ind mL^{-1}, compared to 468.3 and 360.5 ind mL^{-1} at 35 and 15ºC, respectively. Transferring the cultures from 27 to 35ºC significantly increased the rate of resting egg carrying females with the maximum rate of 31.5% compared to 21.2 and 13.5% of the rotifers at 27 and 15ºC, respectively. The resting egg densities of the cultures at 35ºC were also significantly higher than those at 15 and 27ºC. The resting egg carrying females appeared and increased their rates in concurrence with increases in the population density. The present results are important information for resting egg induction and production of rotifer in larviculture.

Keywords

Rotifer, *Brachionus calyciflorus*, resting egg

Introduction

Rotifer has been widely used in aquaculture as a valuable live feed for larvae and fry of most fish species. Their small size and relatively slow movements combined with the features of staying

Received: June 10, 2020
Accepted: October 9, 2020

Correspondence to
dtnhinh@vnua.edu.vn

suspended in the water column, rapid population growth, and high culture density capacity have contributed to their advantages as ideal prey for the larvae stage of aquaculture species (Lubzens *et al*., 1989). Of which, freshwater rotifers have been utilized in seed production of high-value freshwater species such as giant freshwater prawn (New & Valenti, 2008), loach (Wang et al., 2009), catfish (Clarias anguillaris) (Arimoro, 2007), and ornamental fish (Lim & Wong, 1997). More notably, a significant increase in the survival rates of pangasius catfish fry (Tra, Basa) has been observed when they were fed with freshwater rotifer (Vu *et al.*, 2020).

However, during the larviculture period, rotifers are only required at certain stages (often at the first feeding stage) while the continuous maintenance of a rotifer culture is time-consuming and the availability of rotifer stock fluctuates due to their dependence on being collected from ponds or rivers. There is, therefore, an increased interest in the production of resting eggs, which are also called cysts, because they are ideal for long storage and transport, and they can then be used as inoculum for mass culture (Dhert *et al*., 1997). The utilization of large numbers of resting eggs as inoculum for mass culture considerably reduces labour costs and algae production costs for upscaling the stock culture. The use of rotifer resting eggs is also highly recommended to prevent contamination from disease pathogens in the live food pathways. Resting eggs can tolerate a short exposure to disinfectants such as NaOCl and saltwater, and can be simply treated before hatching to ensure the start cultures are free from bacteria and ciliates (Fengqi, 1996).

Under the impacts of certain environmental factors, including changes in water quality parameters, population density, food availability, and endogenous clues, the life cycle of rotifers undergoes sexual reproduction with the appearance of mictic females (Gilbert, 2003; Stelzer & Snell, 2003). If not fertilized within a few hours of hatching, mictic females will produce haploid male eggs. If fertilized by a male, the mictic females will produce diploid resting eggs which are carried on the females (resting eggs bear females) (Yin *et al*., 2016; Snell *et al*., 2019). Resting eggs with a thick wall, are released by their mothers into the water column and fall to the sediment of water bodies (García-Roger *et al*., 2006). They are highly resistant and can remain vital for a long time in adverse environmental conditions (Segers & Chittapun, 2001). In practical conditions, to induce and produce resting eggs of rotifer, multi-stressors are often applied. Although resting egg production of saltwater rotifer (*B. plicatilis*) has been reported in detail, the related works on freshwater rotifer are limited. In Vietnam, no research has been done on the induction of resting eggs of freshwater rotifer (*B. calyciflorus*) so far, despite its increasing role in larviculture. Xi *et al.* (2004) reported the significant influences of both temperature and strain, independently and in interaction, on resting egg production of *B. calyciflorus*. The present research, therefore, aims to induce and produce resting eggs of *B. calyciflorus* with a strain collected in Northern Vietnam through the manipulation of culture temperatures.

Materials and Methods

Source of rotifer

A zooplankton net with a mesh size of 70µm was used to collect the upper sediment layer from outdoor fish tanks in the Northern part of Vietnam. The large particles in the collected sediment were removed using meshes sized from 200-500µm. Resting eggs of rotifers were then separated using a mesh size of 100µm and were checked under a microscope for further manual isolation if necessary.

Resting eggs at the size of 100x160µm (width x height) were black in color and had a black ring around the extra-embryonic space as mentioned by Hagiwara (1995), which differentiates them from amitic eggs (**Figure 1**).

The eggs were stored in 4ºC and dark conditions for 2 months and hatched after being incubated at 27ºC and D:L 0:24 conditions for 24h. The rotifer stock was then upscale cultured at 27ºC in 20L buckets and fed with condensed freshwater microalgae, *Chlorella vulgaris*. No

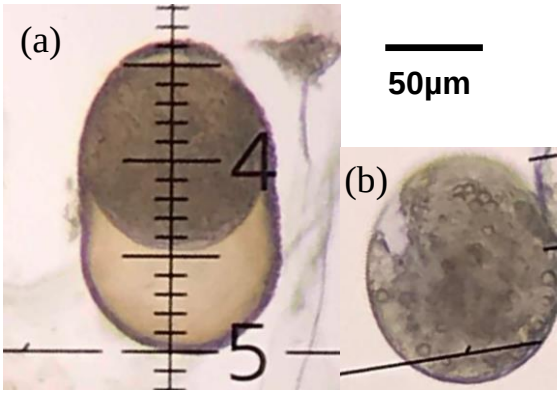

Figure 1. Resting egg of rotifer collected in sediment of fish tanks (a) and an amitic egg (b)

appearance of resting eggs was shown under these culture conditions.

Experimental design

After the pre-culture period at 27ºC, stock rotifers were transferred to the treatment groups at 15 and 35ºC, while the control group was kept at 27ºC. One-litre plastic beakers filled with 500mL culture medium were used to culture the rotifers in the experiment with 3 replications for each temperature treatment. For the treatment at 35ºC, the beakers were placed in a water bath and an electric heater was used to control the temperature. The 27ºC beakers were arranged in an air-conditioned room which was set at 27ºC while the beakers of the 15ºC treatment were placed in a transparent cool refrigerator. All of the treatments were provided with the same light source to reach a light density around 2000 lux and a L:D 18:6 regime.

The rotifers were inoculated at the initial density of 200 ind mL^{-1} into the experimental beakers after an acclimation period of one day to each of the tested temperatures. Weak aeration was applied in all of the culture beakers throughout the experiment period. The rotifers were fed twice daily with condensed fresh *Chlorella vulgaris* at densities from 2 to 5 x 10^6 cells mL^{-1}. By the time of feeding, 10-20% of the culture volume was exchanged daily.

The number of rotifers was counted once daily. A 1ml sample of the culture medium was collected from each culture beaker after increasing aeration for well-mixing of the rotifers in the water column. The samples were inspected and counted daily under microscopes for the total number of rotifers and number of resting egg-bearing females, while the resting egg density was counted on the last day of the experiment. Each culture beaker was sampled 3 times for mean values. The population growth rate (r) of the rotifers was calculated as follows:

$$r = (LnN_T - LnN_0)/T$$

where, T is the culture day in which the rotifer density was the highest, and N_0 and N_T are the initial and highest rotifer densities, respectively (Hagiwara & Hino, 1988). The percentage of resting egg-bearing females w calculated as follows:

Number of resting egg-bearing females/total number of rotifers counted *100%.

The data of the population growth rate, the maximum density of rotifers, the rate of resting egg-bearing females, and resting egg density were subjected to one-way analysis of variance (ANOVA) and if significant ($P< 0.05$) differences were found, Tukey's post-hoc test was used to rank the groups in SPSS version 16.

Results and Discussion

Population growth

Population growth of the rotifers was higher at high temperatures. The maximum density of the rotifers cultured at 15ºC reached 360.5 ind mL^{-1}, significantly lower than those of rotifers kept at 27ºC or exposed to 35ºC ($P< 0.01$). The two later values also significantly differed from each other ($P< 0.01$) with the lower maximum density belonging to the rotifers exposed to 35ºC, 468.3 ind mL^{-1}, compared to 608.3 ind mL^{-1} for

the rotifers cultured at 27ºC. The population growth rate (r-value) of the rotifers shifted to culture at 15ºC was 0.282 and significantly lower ($P< 0.01$) than those of the rotifers cultured at 27 and 35ºC, which reached 0.416 and 0.447, respectively (**Table 1**).

The rotifer population growth curves showed different trends (**Figure 2**). The population density of rotifers cultured at 35ºC increased dramatically from day 2 and reached the highest point on day 5, followed by a rapid reduction period, and no rotifer survived after day 7 of culture. A similar trend of population growth was also observed for the rotifers kept at 27ºC, although, the maximum density peaked on day 6 (648.3 ind mL^{-1}) and decreased to 144 ind mL^{-1} on day 10. The changes in population density of the rotifers exposed to 15ºC differed from the two others with a slow increase during the first 7 days of culture, a slight reduction during days 7-10, and a high density of 246 ind mL^{-1} on the last day (day 10).

Temperature is one critical parameter affecting the biological and growth variables of a species in different ways including thermal optimal ranges and thresholds of a specific strain. Lavens & Sorgeloos (1996) reported the temperature tolerance of *B. calyciflorus* was between 15 and 35ºC. However, the rotifer strain used in the present study survived and reproduced well at these two temperatures. The optimal temperature for a high population density and growth rate in the present study was 27ºC, in agreement with previous studies that reported the optimal temperature for population

Table 1. Population growth of rotifers cultured at 15, 27, and 35ºC

Temperature (°C)	Days of maximum population density	Maximum population density (ind mL^{-1})	Population growth rate (r)
15	7	$360.5^{a} \pm 22.3$	$0.282^{a} \pm 0.011$
27	6	$608.3^{c} \pm 37.4$	$0.416^{b} \pm 0.012$
35	5	$468.3^{b} \pm 28.7$	$0.447^{b} \pm 0.014$
Tukey's test		***	***

*Note: Values followed by different letters in each treatment column are significantly different at the 5% level by Tukey's test. *** denotes the significance level of 1%.*

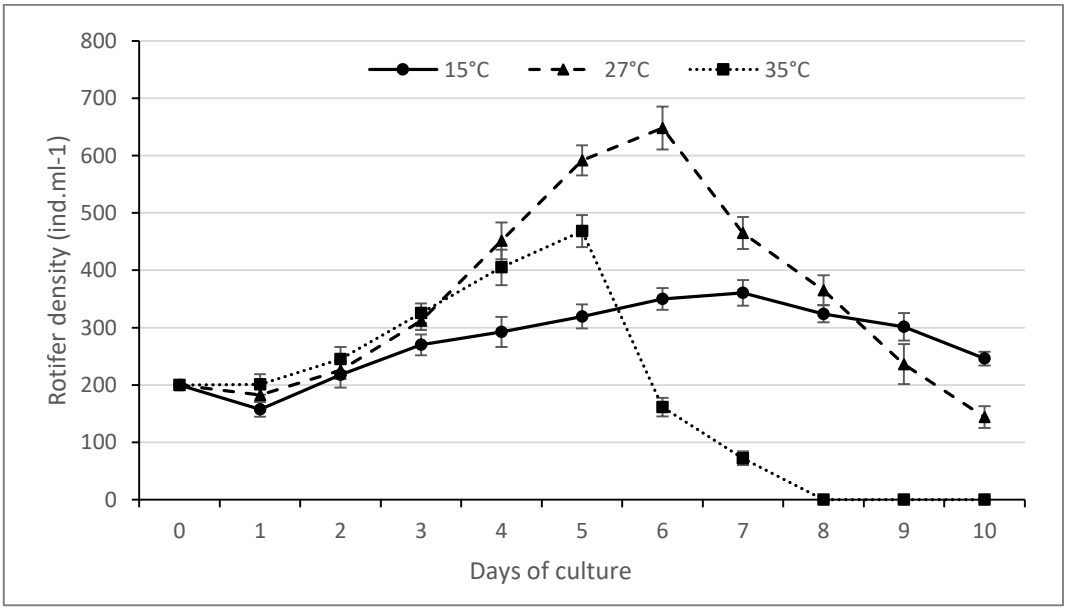

Figure 2. Population growth curves expressed by population density of rotifers cultured at 15, 27, and 35ºC. The data are shown as mean ± standard error (SE),

growth of *B. calyciflorus* was around 25-29ºC (Awaïss & Kestemont, 1992; Park *et al*., 2001; Anitha *et al*., 2016). At high temperatures, the rapid increase in population growth can compromise water quality and inhibit further growth of the population (Ogello *et al*., 2016). This may be also the reason for the rapid decreases in the population densities of the higher temperature groups at the later culture stages in the present study.

An increase in the population growth rate as the water temperature increased was observed due to the shorter time required for the embryo and post-embryo to develop at a higher temperature (Awaïss & Kestemont, 1992). At high temperatures, the metabolic activities of rotifer are high, the lifespan shortens, and the number of rotifer eggs and juveniles released per day increases due to the reduction in the interval between each egg-laying (Awaïss & Kestemont, 1992; Yona, 2018).

Production of resting eggs

Resting egg-bearing females appeared and the rates increased in all of the treatments as the population density increased but were different among the temperature groups (**Table 2, Figures 3 and 4**). On the first day of the experiment, no resting egg-bearing females were observed in any of the temperature treatments. The earliest appearance of resting egg females was detected at 35ºC after one day of the experiment and the rate increased most dramatically to the maximum value of 31.3% on day 5, significantly higher ($P< 0.01$) than those of rotifers kept at 27ºC or exposed to 15ºC. The exposure of rotifers to 15ºC produced resting egg-bearing females on day 3 and the maximum rate reached 13.5% on day 8, while rotifers kept at 27ºC obtained the maximum rate of resting egg-bearing females on day 7.

The density of the resting eggs at the end of the experiment significantly differed ($P< 0.01$) among the treatments, with the highest density reaching 98.3 eggs mL^{-1} in the 35ºC treatment, followed by those in the 27 and 15ºC treatments with 64.6 and 45.5 eggs mL^{-1}, respectively.

Resting egg females typically are found during periods of rapid population growth or high population densities (Carmona *et al*., 1995; Schröder, 2001). The same trends were also observed in the present study with the appearance and increasing rates of resting egg females as the population density increased. The crowding condition was believed to assure a high probability of encounters between males and mictic females, and that guaranteed the

Figure 3. Resting egg females produced in the experiment

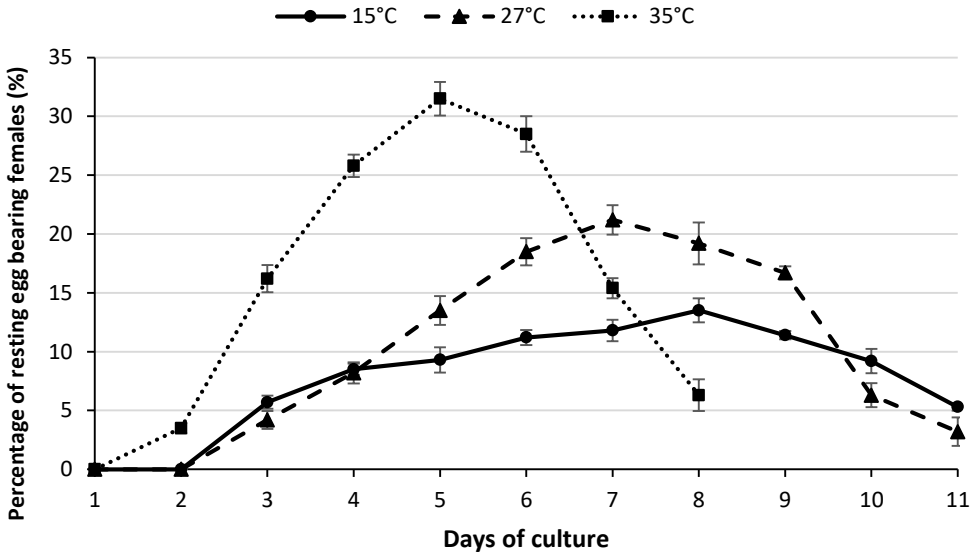

Figure 4. Percentage of resting egg-bearing females cultured at different temperatures

Table 2. The rate of resting egg females and resting egg densities obtained at different temperatures

Temperature (ºC)	Maximum rates of resting egg female (%)	Resting egg density (eggs mL^{-1})
15	$13.5^{a} \pm 1.02$	$45.5^{a} \pm 6.8$
27	$21.2^{b} \pm 1.15$	$64.6^{b} \pm 5.8$
35	$31.5^{c} \pm 1.43$	$98.3^{c} \pm 10.7$

Note: The data are shown as mean ± SE. The values with different letters in the same column are significantly different (P< 0.05).

production of large numbers of resting eggs (Gilbert, 2003). Crowding was reported in various previous studies to induce production of mictic females in *B. calyciflorus* (Gilbert, 2003) and *B. plicatilis* (Hino & Hirano, 1976; Snell & Boyer, 1988; Stelzer & Snell, 2003). Induction of mictic females in rotifers of the genus *Brachionus* is believed to be stimulated by a chemical that is released into the water and accumulated at high population densities (Stelzer & Snell, 2003). However, threshold densities vary among species and probably among strains and clones of a species (Gilbert, 2007). For the strain used in the present study, no resting egg females were found when the population density was below 200 ind.ml^{-1} in all of the temperature treatments.

The present results showed that rotifers exposed to a high temperature (35ºC) obtained higher resting egg production compared to those of rotifers kept at a culturing temperature of 27ºC or exposed to 15ºC. From previous reports, the induction of rotifer resting eggs has been shown to be species-dependent with temperature. Xi *et al*. (2004) reported the significant effect of temperature on resting egg production of the rotifer strain *B. calicyflorus* with significantly higher resting egg production at 30ºC compared to those of rotifers at 20 and 25ºC. However, Xi *et al*., (2004) observed elevated rates of resting egg females at 20ºC in comparison to those of rotifers at 25 and 30ºC in another strain of *B. calyciflorus*. Assavaaree *et al.* (2003) showed that a significantly higher number of resting eggs were produced when the rotifer *B. rotundiformis* (Langkawi, Japan strain) was transferred from 30ºC to 25ºC compared to that of the control treatment at 25ºC. In other studies, although a Hawaiian strain of *B. rotundiformis* produced resting eggs at a high temperature (30ºC) (Hagiwara *et al*., 1989; Hagiwara *et al*., 1991), the Koshiki strain

produced resting eggs at a slightly higher temperature (35ºC). In a study by Hagiwara *et al*. (1995), mictic induction was observed only at 25ºC in *B. plicatilis*, but for *B. rotundiformis*, it increased as the temperature increased and the highest percentages were at 35ºC compared to at 25 and 30ºC.

Conclusions

Increased temperature significantly enhanced the population growth rate and the maximum density of the rotifer strain *Brachionus calyciflorus* used in the present study. The exposure of this rotifer strain to a high temperature (35ºC) elevated resting egg production compared to that of rotifers kept culturing at 27ºC or exposed to 15ºC.

References

Anitha P. S., Sabu A. S. & George R. M. (2016). Reproductive rate of *Brachionus calyciflorus* under the influence of salinity, temperature, feed type and feed concentration. International Journal of Fisheries and Aquatic Studies. 4: 219-226.

Arimoro F. (2007). First Feeding in the African Catfish *Clarias anguillaris* Fry in Tanks with the Freshwater Rotifer *Brachionus calyciflorus*. Journal of Fisheries and Aquatic Science. 2(4): 275-284.

Assavaaree M., Hagiwara A., Kogane T. & Arimoto M. (2003). Effect of temperature on resting egg formation of the tropical SStype rotifer *Brachionus rotundiformis* Tschugunoff. Fisheries Science. 69(3): 520-528.

Awaïss A. & Kestemont P. (1992). An investigation into the mass production of the freshwater rotifer *Brachionus calyciflorus* Pallas. 2. Influence of temperature on the population dynamics. Aquaculture. 105(3-4): 337-344.

Carmona M. J., Gómez A. & Serra M. (1995). Mictic patterns of the rotifer *Brachionus plicatilis* Müller 1786 in small ponds. Springer, Dordrecht. In Rotifera VII: 365-371.

Dhert P., Schoeters K., Vermeulen P., Sun J., Gao S., Shang Z., Naihong X., Van Duffel H. & Sorgeloos P. (1997). Production, disinfection and evaluation for aquaculture applications of rotifer resting eggs from Bohai Bay, PR of China. Aquaculture International. 5(2): 105-112.

Fengqi L. (1996). Production and application of rotifers in aquaculture. Aquaculture Magazine. 22(3): 16-22.

García-Roger E. M., Martínez A. & Serra M. (2006). Starvation tolerance of rotifers produced from parthenogenetic eggs and from diapausing eggs: a life table approach. Journal of Plankton Research. 28(3): 257-265.

Gilbert J. J. (2007). Timing of diapause in monogonont rotifers: mechanisms and strategies. In Diapause in Aquatic Invertebrates Theory and Human Use. Springer, Dordrecht: 11-27.

Gilbert J. J. (2003). Specificity of crowding response that induces sexuality in the rotifer Brachionus. Limnology and Oceanography. 48: 1297-1303.

Hagiwara A., & Hino A. (1989). Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer *Brachionus plicatilis*. Springer, Dordrecht. In Rotifer symposium V: 415-421.

Hagiwara A., Kotani T., Snell T. W., Assava-Aree M. & Hirayama K. (1995). Morphology, reproduction, genetics, and mating behavior of small, tropical marine *Brachionus* strains (Rotifera). Journal of Experimental Marine Biology and Ecology. 194(1): 25-37.

Hagiwara A., Lee C. S., Miyamoto G. & Hino A. (1989). Resting egg formation and hatching of the S-type rotifer *Brachionus plicatilis* at varying salinities. Marine Biology. 103(3): 327-332.

Hagiwara A., Lee C. S., Miyamoto G. & Hino A. (1991). Resting egg formation of the L-and S-type rotifer *Brachionus plicatilis* under different water temperature. Nippon Suisan Gakkaishi. 57(9): 1645-1650.

Hino A. & Hirano R. (1976). Ecological studies on the mechanism of bisexual reproduction in the rotifer *Brachionus plicatilis*. I. General aspects of bisexual reproduction inducing factors. Bulletin of the Japanese Society for the Science of Fish. 42(10): 1093-1099.

Lavens P. & Sorgeloos P. (1996). Manual on the Production and Use of Live Food for Aquaculture. Rome: FAO, 375 pp.

Lim L. C. & Wong C. C. (1997). Use of the rotifer, *Brachionus calyciflorus* Pallas, in freshwater ornamental fish larviculture. Hydrobiologia. 358(1-3): 269-273.

Lubzens E., Tandler A. & Minkoff G. (1989). Rotifers as food in aquaculture. Hydrobiologia. 186(1): 387-400.

New M. B. & Valenti W. C. (2008). Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*. John Wiley & Sons.

Ogello E. O., Kim H. J., Suga K. & Hagiwara A. (2016). Lifetable demography and population growth of the rotifer *Brachionus angularis* in Kenya: influence of temperature and food density. African Journal of Aquatic Science. 41(3): 329-336.

Park H. G., Woo K. L., Cho S. H., Kim H. S., Jung M. M. & Kim H. S. (2001). High density culture of the freshwater rotifer, *Brachionus calyciflorus*. Springer, Dordrecht. In Rotifera IX: 369-374.

Schröder T. (2001). Colonising strategies and diapause of planktonic rotifers (Monogononta, Rotifera) during aquatic and terrestrial phases in a floodplain (Lower

Oder Valley, Germany). International Review of Hydrobiology: A Journal Covering all Aspects of Limnology and Marine Biology. 86(6): 635-660.

Segers H. & Chittapun S. (2001). The interstitial Rotifera of a tropical freshwater peat swamp on Phuket Island, Thailand. Belgian Journal of Zoology. 131: 25-31.

Snell T. W. & Boyer E. M. (1988). Thresholds for mictic female production in the rotifer *Brachionus plicatilis* (Muller). Journal of Experimental Marine Biology and Ecology. 124(2): 73-85.

Snell T., Walsh, E., Sarma, S. S.S. & Segers H. (2019). Phylum Rotifera. 10.1016/B978-0-12-385024-9.00008-3.

Stelzer C. P. & Snell T. W. (2003). Induction of sexual reproduction in *Brachionus plicatilis* (Monogononta, Rotifera) by a density-dependent chemical cue. Limnology and Oceanography. 48(2): 939-943.

Vu N. U., Pham T. H., Huynh P. V., & Huynh T. G. (2021). Importance of the freshwater rotifer *Brachionus angularis* for improved survival rate of early life-history stages of pangasius catfish, *Pangasianodon hypophthalmus*. Aquaculture Research. 52(2): 783-792.

Wang Y., Hu M., Wang W. & Cao L. (2009). Effects on growth and survival of loach (*Misgurnus anguillicaudatus*) larvae when co-fed on live and microparticle diets. Aquaculture Research. 40(4): 385-394.

Xi Y. L., Dong L. L., Ge Y. L. & Liu G. Y. (2004). Resting egg production of *Brachionus calyciflorus* (Rotifera) at different water temperatures. Journal of Freshwater Ecology. 19(4): 567-573.

Yin X. W., Tan B. B., Zhou Y. C., Li X. C. & Liu W. (2016). Development time of male and female rotifers with sexual size dimorphism. Hydrobiologia. 767(1): 27-35.

Yona G. K. (2018). Effect of culture temperature on rotifers (*Brachionus plicatilis* sensu strictu) size and reproduction activities. Tanzania Journal of Agricultural Sciences. 17(1): 23-30.