Effect of Culture Temperature on Rotifers (*Brachionus plicatilis sensu strictu*) Size and Reproduction Activities

Yona, G.K.

Tanzania Fisheries Research Institute
P.O. Box 9750, Dar es Salaam
Email: *glokavia@yahoo.com; gloriayona@tafiri.go.tz,*
Mobile: +255 763 100066

Abstract

Genus Brachionus of Rotifers is generally used as a live food during the rearing of fish larvae in early stages. However there is limited information on how culture temperature can affect the size and reproduction of rotifers. In this study Brachionus plicatilis sensu strictu (s.s) was selected as a model organism. Ten rotifers were replicated ten times and cultured at stocking density of 1 rotifer/ml at three different temperature 8°C, 21°C and 30°C. Rotifers were fed Chlorella sp. at a level of 1.5 X 10^6 cells $rotifer^{-1}$. Temperature showed a significant effect on rotifers' size and rotifers' length ranged from 169 to 275µm. There was also a significant ($p<0.001$) effect of culture temperature on rotifers lifespan. These results indicate that low temperature prolongs juvenile period and therefore more materials are allocated to body growth. Metabolic activities are high at high temperature and shorten lifespan. The culture temperature showed a significant ($p<0.03$) effect on number of eggs and juveniles/day. This study has demonstrated that culture temperature has an influence on rotifer's (B. plicatilis s.s.) size and reproduction activities.

Key words: Rotifers, Culture Temperature, Size, Reproduction

Introduction

Rotifers of genus *Brachionus* are widely used as a live food for fish larvae during the early stages of life. *Brachionus plicatilis* is used to feed more than 60 species of marine finfish species and 18 species of crustaceans (Abu-Rezq *et al.,* 2002). Rotifers are used in hatchery due to its high nutritional quality, body size, relative slow swimming velocity and ability to stay in suspended in water column. More than that rotifers are tolerance to a wide range of environmental condition, high reproductive rate and can be enriched with fatty acid, antibiotics and or probiotichs which can be transferred to the final predator.

At present, the genus *Brachionus* are available in three major types including *B. plicatilis* (the large type), the *B. rotundiformis* (small type) and the *B. rotundiformis* ss (super small type) and this classification has been based on morphological and ecological differences (Serra *et al.,* 1998). Population and molecular genetic studies have revealed that the *B. plicatilis* group is further divided and is made up of the genus *B. plicatilis sensu strictu*, *B. austria*, *B. manjavacas* and *B. nevada* (Ciros-Perez *et al.,* 2001).

Many factors influence the morphological and life parameters in rotifers. Body size and age at maturity have been considered as a result of natural selection. However, the extent to which these parameters vary is difficult to predict. It is indicated in earlier studies that feed and temperature levels have influence on somatic cells growth and age at maturity of *Brachionus patulus* (Sarma, 1989) and *Synchaeta pectinata* (Stelzer, 2002). Atkson, (1994) suggested that body size increases at low temperature. Fish larvae fed on prey of specific body size due to different mouth gap/opening. Despite the development in research regarding rotifers, not much has been done with the *B. plicatilis* s.s. Therefore, this study was designed to investigate the effect of temperature on *B. plicatilis* s.s. size and reproduction activities.

Materials and Methods

Rotifers culture and experimental set up

Brachionus plicatilis ss. (clone 10) with amictic eggs were used in this study. The rotifers stock was maintained under controlled culture conditions of 28°C, 25 ppt salinity and 2000 lux light intensity. Rotifers were fed with Chlorella sp. at a density of 1.5 x 10^6 cell/ml. The culture procedure has been described by Dhert. (1996). Three temperatures namely 30°C, 21°C and 8°C were employed in this study. To warm the water to 30°C and 21°C submerged thermostats heaters were used and air stones were used to mix water in the water bath. The low temperature, 8°C was obtained by using cooling recirculation system. Ten *B. plicatilis* s.s. neonates (of nearly the same age) were obtained from a large quantities of amictic eggs. The neonates were introduced into a vial of 10 ml at a density of 1 individual/ml at the start and were placed in the three experimental temperatures. Each temperature was replicated 10 times. During the experiment individual rotifers were transferred daily into a new feed suspension until they died. Rotifer size was determined by measuring the lorica length, using microscope with an eye piece ruler. A total of 10 rotifers were measured and recorded daily until no further change in the lorica length was observed.

The hatching percentage was calculated by the following formula:

$$\text{Hatching (\%)} = \left(\frac{\text{number of juvenile hatched}}{\text{number of eggs incubated}} \right) * 100$$

The life span of the rotifers, in each vial was recorded from the day of hatch to the day of death. The reproductive period i.e. pre-reproduction, reproduction and senile period was also recorded in each treatment. The rotifers' reproduction output is the summation of the total number of juveniles plus loose eggs in the vial for the whole reproductive period divide by the initial number of rotifer in each treatment.

Statistical Analysis

The rotifer size, age at first egg and life span of the rotifers were compared statistically using one-way Analysis of Variance (ANOVA) between treatments with STATISTICA software at significant level of 0.05.

Results

Rotifer size

Rotifers and egg size

The rotifer, *B. plicatilis* s.s. had significantly different size when culture at different temperatures. Rotifers cultured at low temperature 8°C after day five were significantly larger ($p<0.05$) than those cultured at higher temperature fed the same amount and type of feed (figure 1). Rotifers cultured at 20 and 30°C showed no significant different ($p > 0.05$) in size between day one and five of monitoring period and at that period were large in size than those cultured at low temperature, 8°C.

In general, lorica length of rotifers ranged from 169 to 270µm, the largest mean rotifer size at higher temperature, 20 and 30°C were 247µm at day four and 245µm at day five respectively. The largest mean rotifer size at 8°C was 270µm reached at day seven (Figure 1).

Reproduction activities

Lifespan period of rotifer at different culture temperatures

The rotifers cultured at 30°C had the shortest lifespan, which was ten days, while rotifers culture at 21°C which was 13 days whereas rotifers cultured at 8°C had the longest lifespan of 20 days (figure 2).

Juvenile, reproductive and senile period

Rotifers cultured at high temperature, 30°C, had a significantly ($p<0.001$) shorter juvenile period of 12h whereby 95% of all rotifers had eggs at that particular time. Rotifers culture at 21°C, has long juvenile period as 100% rotifers had no eggs at 24h while rotifers cultured at 8°C had longest juvenile period of 120h all rotifers had no eggs.

The rotifers cultured at 21°C, had the longest reproductive period of 11 days followed by rotifers cultured at 30°C, which was nine days whereas the shortest reproductive period was shown by rotifers cultured at 8°C, which was six days. At low temperature 8°C it was difficult to

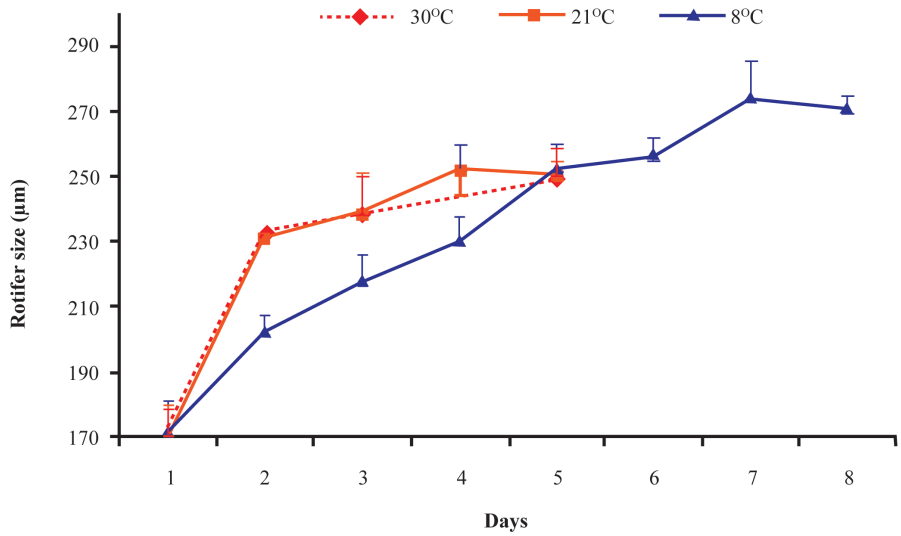

Fig. 1: Size of Rotifers (µm) at each different culture temperatures. Top bars indicate standard deviation. (21°C and 30°C n=50♀; 8°C n=80♀)

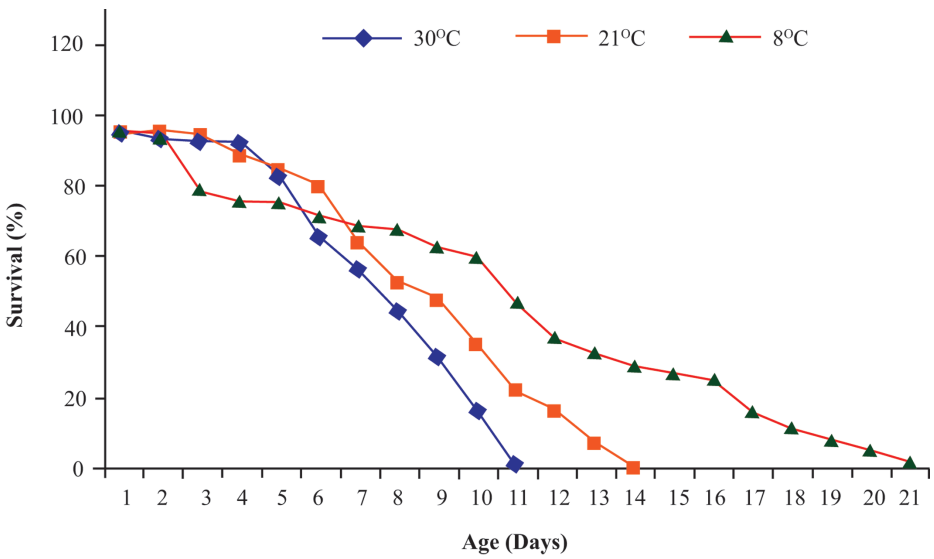

Figure 2: Survival percentage of rotifers cultured at different temperatures (Start n=100 at each temperature).

demarcate the juvenile and reproductive period, as only a small percentage of rotifers carried eggs at that time.

The senile period of rotifers cultured at 30°C started from day 7 - 9, some rotifers had no eggs from day 7 and some rotifers died carrying eggs. The senile period of rotifers cultured at 21°C was longer (started from day 7 – 12) compared to those cultured at 30°C. Some rotifers had no egg from day 7 and some died while carrying eggs. The senile period was longest 10 days ranged from days 11 – 20 for rotifers culture at low temperature 8°C, all rotifers at this period had no eggs until they died.

Egg ratio day^{-1} (eggs rotifer^{-1}day^{-1}) and number of eggs at three culture temperatures

Rotifers cultured at 30°C and 21°C had a maximum 3.64 and 3.67 of egg ratio/day

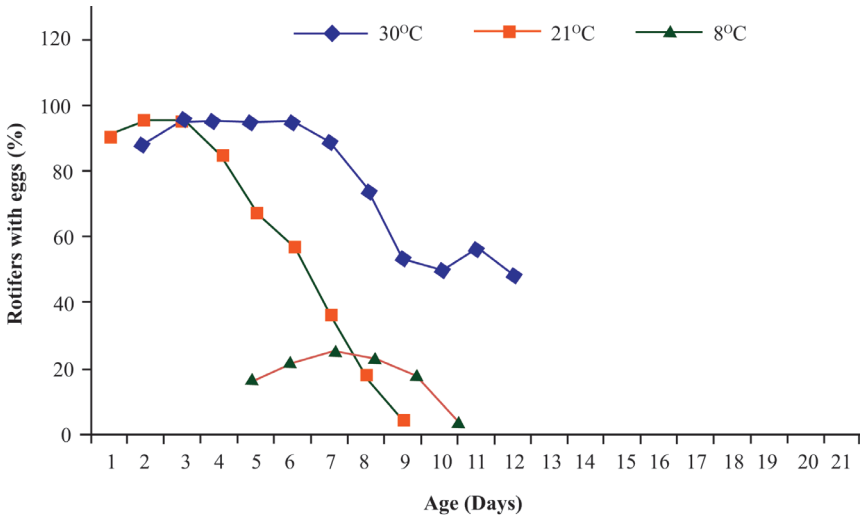

Figure 3: Percentage of rotifers with eggs at different culture temperature (Start n=100♀at each temperature with 10 replicates) Percentage of rotifers with eggs is significantly ($p<0.05$) different temperatures (one way ANOVA)

respectively on day four of the experiment. Rotifers cultured at 8°C had the lowest egg ratio/day of maximum 0.23 on day eight of the experiment. There was no significant ($p>0.07$) difference at maximum egg ratio/day when rotifers were cultured at 30°C or at 21°C. The numbers of eggs carried by rotifers cultured at 30°C ranged from 1-6 eggs rotifer^{-1}, at 21°C it ranged from 1-5 eggs rotifer^{-1}, while for those cultured at 8°C, it was only 1 egg rotifer^{-1} (figure 4).

Juvenile ratio and number of juvenile at different culture temperatures

The average number of juvenile/rotifer/day followed the same trend of eggs/rotifer/day. Rotifers cultured at 30°C produced an average of 14.4 juveniles/rotifer for the whole reproductive period which was significantly ($p<0.0001$) higher than those cultured at 21°C which was 7.74 juveniles/rotifer and for rotifers cultured at 8°C had average of 0.14 juvenile/rotifer for the whole reproductive period. The juvenile/

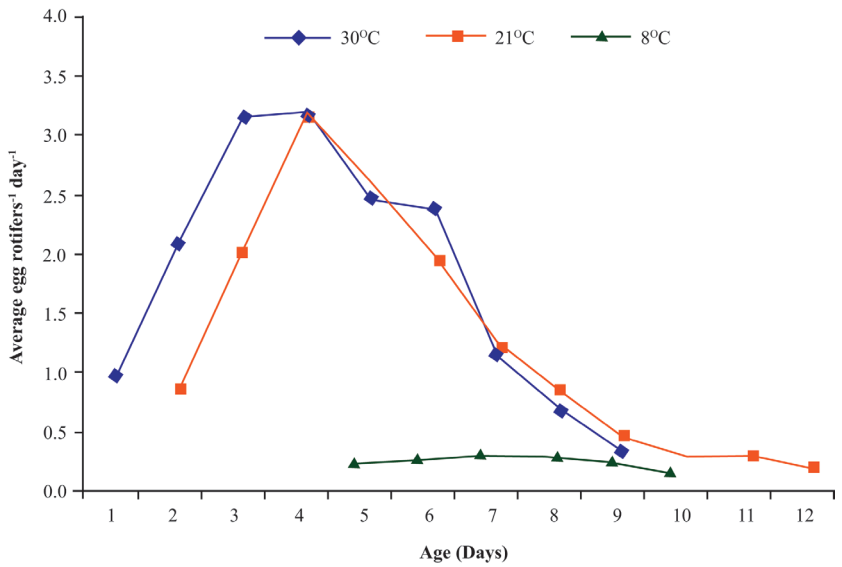

Figure 4: Average egg ratio/day at different temperature (start n = 100♀ at each temperature with 10 replicates)

rotifer/day for rotifers cultured at 30°C reached maximum of 3.53 juveniles at day four whereas rotifers cultured at 21°C, juvenile/rotifer/day reached maximum 2.96 on day 5 and 0.08 on day 8 for rotifers cultured at 8°C temperatures (figure 5).

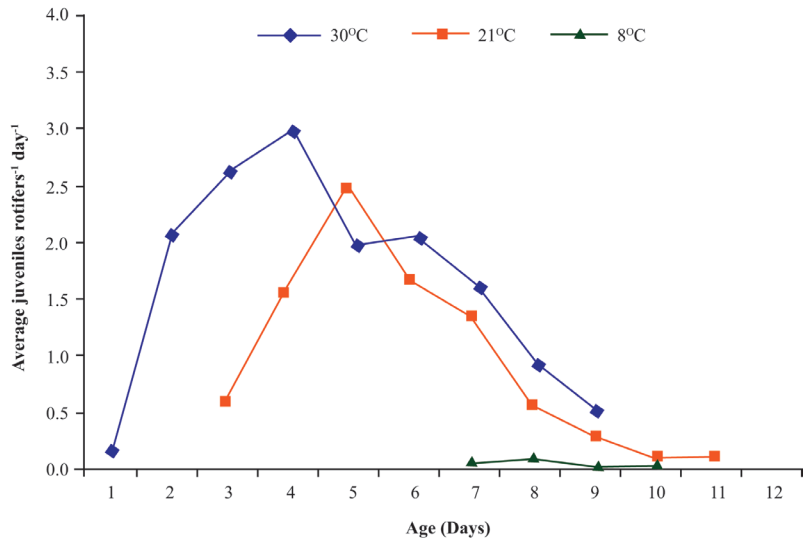

Figure 5: Average juvenile/rotifer/day at different temperature (start n = 100♀ at each temperature with 10 replicates)

Discussion

Lorica length of rotifer *B. plicatilis* has been studied elsewhere, the total lorica length ranging from 171 to 277 µm (Anitha and George, 2006). Values obtained during the present study fall within this range. According to Fukusho and Iwamoto, (1981), rotifer grows in an allometric manner and most of the growth occurs during the embryonic stage. During this stage, certain parts of the body increase in length and size while others stay constant. This study has indicated a significant difference in lorica length between rotifers of the same clone cultured at different temperatures. This suggests that, temperature has influence on the size of rotifer. Specifically, low temperature shown a prolonged juvenile period compared to the temperature despite provision of the same type and amount of feed. Earlier studies have shown that more nutrients are allocated to body growth in different species of rotifers including *Brachionus patulus* and *Synchaeta pectinata* especially when temperatures are lower. In general, most authors on this subject agree that larger rotifers are frequently found at low temperatures (Sarma, 1989; Atkison, 2001; Stelzer, 2002). Similarly, rotifers collected during winter have larger loricae than those collected in summer (Hillbricht-Ilkowska, 1983).

It has been reported that the somatic growth of *B. patulus* occurred only until age at first reproduction and increase in body size if any, during the reproduction period is insignificant, (Sarma, 1989). However, in the present study *B. plicatilis* s.s. cultured at higher temperature continued to grow after the age of first egg. The reason for this might be the high feed levels and therefore intake of energy from feed was in excess of metabolic requirements and egg productions and therefore used for somatic growth. This phenomenon of plasticity leads to the possibility that body size of rotifer can be manipulated by culture conditions. Body size is one of the rotifer characteristics that is considered as a critical feature and determines their adequacy as feed for young fish larvae (Inneke *et al.,* 1998).

Regarding lifespan, it has been suggested that the variations are the consequence of metabolic rate augmentation (Miracle and Serra, 1989; Serra *et al.,* 1994). The shorter lifespan at high

temperature has been reported. For instance, *B. plicatilis* reported a lifespan of 10 days when cultured at 20°C and 7 days when cultured at 25°C and for *S. litorallis* had a lifespan of 5.6 days when cultured at 20°C and 4.4 at 25°C. The difference of lifespan between *B. plicatilis* and *B. plicatilis* s.s. might be due to culture conditions and species differences. The juvenile period was generally subjected to maximum environmental influence. It is only during this period that somatic growth takes place because of active feeding, since all cell divisions are completed before hatching. The somatic growth of rotifer is mainly because of assimilated material (Sarma, 1989). Results of this study suggest that at high temperature rotifers are more active feeders than at low temperature. The same trend was reported *B. patulus* that high temperature shortens juvenile period (Sarma, 1989).

It was also shown that, reproductive period of *B. plicatilis* s.s. was highly affected by temperature. 30°C shortens the juvenile period, increases intrinsic growth rate and shortens lifespan, all this was reported as a consequence of metabolic rate increase (Miracle and Serra, 1989; Serra *et al.,* 1994). At low temperatures, rotifers take a longer egg production time to produce egg cell materials. Since a lot of energy is spent for growth, this makes the reproductive period short, before the rotifer enters the senile period. This strain of *B. plicatilis* exhibited slower growth and development rates reduced reproductive rates, longer life span, and larger body size at lower temperature (Nagata, 1985). The senile period in the present study shows a similar trend, and suggests the same reasons to explain this.

The egg ratio/day at high temperature was higher than at low temperature. It has been reported that temperature affects the rate of egg development (Duncan and Gulati, 1983) and rates of biochemical reactions such as metabolism, feeding, movement, longevity, as well as reproduction (Sarma and Rao, 1990). However, in the present study, results show that rotifers cultured at high temperature had high egg ratio/day compared to those cultured at low temperature, this suggests that energy intake was high enough to cover metabolic activities and the portion for reproduction unlike for cold temperature. The egg ratio/day has been used as a predictor of population growth in the culture. Egg ratios typical of exponentially growing *B. plicatilis* populations at 25°C ranged from 0.5-1.2 (Snell *et al.,* 1987) and rotifer populations reproducing at a replacement level (stationary phase) had egg ratios between 0.13-0.5. Once egg ratio fell below 0.13, populations declined (Liu, 1996). However, present results of this study do not concur to the findings above, on the exponential phase the egg ratio day-1 was as high as 2.2 for 30°C and 21°C and for 8°C was below the ratio reported, this suggests that *B. plicatilis* s.s. is a different species which behave differently from the *B. plicatilis* group. Rotifers cultured at 8°C had overall the lowest egg ratio when compared to other culture temperatures; this shows that reproduction in *B. plicatilis* s.s. is highly influenced by temperature, the lower the temperature the lower the reproductive rate.

In the present study, juvenile/rotifer/day produced by *B. plicatilis* s.s. followed a similar trend of eggs/rotifer/day. More juveniles/rotifer/day at higher temperatures than lower temperatures, was due to higher amount of eggs spawned at higher temperature as compared to eggs produced at low temperature. The same reasons mentioned above are explaining the difference in juveniles/rotifer/day. The average number of juveniles for the whole reproductive phase at 30°C was approximately double of that at 21°C. The reason for this might be, as reported, that rotifers at higher temperature grow quickly, reproduces fast and die faster than those rotifers cultured at low temperature (Serra *et al.,* 1994). The number of juveniles/rotifer^{-1} at 8°C was the lowest, this shows that the population growth is negative, meaning that the number of juveniles hatched were lower than the number of adults' dies. These results are concurred with Lui (1995) that with the egg ratio below 0.13, the population is declining.

Conclusion

We concluded that temperature influences the rotifer *B. plicatilis* s.s. body size. Rotifers

grew bigger at lower temperature and has long lifespan. Lower temperature delays egg production, prolongs juvenile period and the senile period with shorter reproductive period. Higher temperature results in faster reproduction with high egg ratio, many juvenile per rotifer and small body size. Therefore, for quick, large number and small size of *B. plicatilis* s.s. production for fish larvae then opt for high culture temperature.

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