The Effect of Food Concentration on the Life History of Three Types of *Brachionus calyciflorus* Females

**key words:** rotifera, amictic and mictic females, juvenile, reproductive and post-reproductive period, eggs, *Scenedesmus obliquus*

**Abstract**

The effect of food concentration on the life history of three types of *Brachionus calyciflorus* females (amictic, unfertilized mictic and fertilized mictic female) was studied with replicated individual cultures at 25 ° and at four food concentrations (1.5, 3.0, 6.0 and 9.0 × 10⁶ cells mL⁻¹) of *Scenedesmus obliquus*. There were highly significant effects of both food concentration and female type, independently and in interaction on the duration of juvenile period of the rotifer, but neither were the effects on the duration of post-reproductive period and mean life-span. The duration of juvenile period of unfertilized mictic female at the food concentration of 9.0 × 10⁶ cells mL⁻¹ was the longest among all the food concentration-female type combinations. Both food concentration and female type influenced significantly the duration of reproductive period and the number of eggs produced by each type of female per life cycle, respectively. There was, however, no significant interaction between food level and female type. Among the three types of females, the number of eggs produced by an unfertilized mictic female was the largest, and that of a fertilized mictic female was the smallest.

**1. Introduction**

Food quantity is one of the most important factors that influence the life history characteristics and population dynamics of rotifers (Schmid-Araya, 1991). Some studies examined the effect of food concentration on the duration of the phases of life cycles in amictic females (King, 1967; Halbach and Halbach-Keup, 1974; Pilarska, 1977; Robertson and Salt, 1981; Schmid-Araya, 1991; Guisande et al., 1993; Xi and Huang, 1999). Little is known about the effect of food concentration on life history of mictic females. Pourriot (1973) studied the mean life-span and net reproduction rate of amictic and unfertilized mictic females of six species of rotifers including *Brachionus calyciflorus* (Pallas). Snell (1986) studied the effects of temperature, salinity and food level on the population growth, mean life-span and fecundity of amictic and unfertilized mictic females of *B. plicatilis* (O. F. Müller). Snell and Boyer (1988) reported the thresholds including food concentration for mictic female production in *B. plicatilis*. Galindo and Guisande (1993) have investigated the effect of food quantity on life history of unfertilized mictic females in *B. calyciflorus* and compared their data with other related papers to find whether differences between life history parameters of unfertilized mictic females and those of amictic females generally exist. Snell and Carmona (1995) reported a different toxicant sensitivity of sexual and asexual reproduction in *B. calyciflorus*. Clearly, more work on the population dynamics of
amictic, unfertilized mictic and fertilized mictic females need to be done. Such studies can not only accumulate material for investigating physiological ecology of the three types of rotifer females and explaining the ecological mechanism of rotifer resting egg formation (POURRIOT and SNELL, 1983), but also are important for the mass production of rotifers or their resting eggs. Thus, the following study was designed to investigate the effect of food concentration on life history characteristics of three types of *B. calyciflorus* females.

2. Materials and Methods

*Brachionus calyciflorus* was obtained by hatching resting eggs collected from sediments of Lake Donghu and thereafter clonal culturing. Stock rotifer cultures were kept at 27 ± 1 °C on a 16: 8h light: dark photoperiod at 130 lx provided by a fluorescent light in a thermostat water bath. Rotifer cultures were daily fed on *Chlorella pyrenoidosa* (CHICK.) Before the experiment commenced, the rotifer cultures were fed on four levels (1.5, 3.0, 6.0 and 9.0 × 10⁶ cells mL⁻¹) of *Scenedesmus obliquus* (KÜTZ.) at 25 ± 1 °C for at least two weeks.

Algae were grown in a semi-continuous culture using HB-4 medium (LI et al., 1959) renewed daily at 40%. Algae in exponential growth were concentrated by centrifuging and resuspended in the rotifer medium (GILBERT, 1963). Algal concentrations were measured with a hemacytometer and diluted to the desired experimental food concentrations.

Approximately 100 animals with amictic eggs were randomly removed from each stock culture and individually placed into 1.5 mL rotifer medium of each food concentration on 24-well plexiglass tissue culture plates. About half of these animals were individually cultured and the others were cultured with 2–3 2h-old males. All these cultures were observed every 1 –2 h under a Wild dissecting microscope at 25× magnification over a 10 h period. The time of birth of the offspring was recorded, and each neonate was transferred to a new well. On the following day, once the neonates reached maturity, 10 amictic, unfertilized mictic and fertilized mictic females were randomly selected, respectively. Thereafter,

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Female type</th>
<th>9.0 × 10⁶ cells mL⁻¹</th>
<th>6.0 × 10⁶ cells mL⁻¹</th>
<th>3.0 × 10⁶ cells mL⁻¹</th>
<th>1.5 × 10⁶ cells mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of juvenile period</td>
<td>A.F</td>
<td>31.50 ± 2.95</td>
<td>19.00 ± 2.54</td>
<td>19.60 ± 4.88</td>
<td>23.80 ± 6.34</td>
</tr>
<tr>
<td></td>
<td>U.M.F</td>
<td>21.40 ± 6.04</td>
<td>18.70 ± 1.34</td>
<td>16.55 ± 4.46</td>
<td>18.90 ± 2.60</td>
</tr>
<tr>
<td></td>
<td>F.M.F</td>
<td>41.70 ± 6.07</td>
<td>27.65 ± 1.83</td>
<td>26.80 ± 1.48</td>
<td>29.85 ± 2.75</td>
</tr>
<tr>
<td>Duration of reproductive period</td>
<td>A.F</td>
<td>27.60 ± 48.36</td>
<td>42.40 ± 8.75</td>
<td>56.20 ± 11.58</td>
<td>53.60 ± 25.53</td>
</tr>
<tr>
<td></td>
<td>U.M.F</td>
<td>43.10 ± 19.22</td>
<td>45.40 ± 23.27</td>
<td>39.40 ± 24.53</td>
<td>53.50 ± 24.05</td>
</tr>
<tr>
<td></td>
<td>F.M.F</td>
<td>18.40 ± 21.63</td>
<td>39.90 ± 10.55</td>
<td>41.90 ± 18.08</td>
<td>32.10 ± 23.53</td>
</tr>
<tr>
<td>Duration of post-reproductive period</td>
<td>A.F</td>
<td>31.10 ± 12.57</td>
<td>31.20 ± 11.27</td>
<td>29.70 ± 12.61</td>
<td>30.50 ± 11.20</td>
</tr>
<tr>
<td></td>
<td>U.M.F</td>
<td>44.90 ± 35.43</td>
<td>25.10 ± 13.91</td>
<td>49.80 ± 31.85</td>
<td>31.00 ± 16.53</td>
</tr>
<tr>
<td></td>
<td>F.M.F</td>
<td>42.95 ± 23.67</td>
<td>36.90 ± 16.93</td>
<td>35.30 ± 12.73</td>
<td>36.00 ± 29.48</td>
</tr>
<tr>
<td>Life-span</td>
<td>A.F</td>
<td>90.20 ± 56.62</td>
<td>92.60 ± 16.45</td>
<td>105.50 ± 10.27</td>
<td>107.90 ± 23.97</td>
</tr>
<tr>
<td></td>
<td>U.M.F</td>
<td>109.40 ± 36.90</td>
<td>89.20 ± 21.28</td>
<td>105.75 ± 32.06</td>
<td>103.40 ± 25.76</td>
</tr>
<tr>
<td></td>
<td>F.M.F</td>
<td>103.05 ± 35.44</td>
<td>104.45 ± 22.40</td>
<td>104.00 ± 12.79</td>
<td>98.05 ± 27.53</td>
</tr>
<tr>
<td>Number of eggs</td>
<td>A.F</td>
<td>3.30 ± 3.40</td>
<td>6.50 ± 1.84</td>
<td>6.80 ± 1.48</td>
<td>5.20 ± 2.04</td>
</tr>
<tr>
<td></td>
<td>U.M.F</td>
<td>13.90 ± 5.36</td>
<td>17.30 ± 5.06</td>
<td>12.70 ± 5.85</td>
<td>12.10 ± 5.45</td>
</tr>
<tr>
<td></td>
<td>F.M.F</td>
<td>1.80 ± 0.92</td>
<td>3.10 ± 0.57</td>
<td>2.70 ± 0.48</td>
<td>2.30 ± 0.82</td>
</tr>
</tbody>
</table>

Observations were made every 2–6 h for the occurrence of eggs and neonates, which were counted and removed. Rotifers were transferred to new media with corresponding concentration of food suspension daily, respectively. Between observations, the culture plates were placed in an illumination incubator at 25 ± 1 °C. Light intensity was approximately 3000 lx (16L: 8D). These observations continued until the rotifers died, the time of death of each animal being recorded.

Regression analysis of the relationship between each of the life history parameters and the food concentrations was done with the statistical packages (Statgraphics, Statistical Graphics Corp., 1987). Two-way analysis of variance (ANOVA) was conducted to identify significant effects of food level, female type as well as food level × female type interaction on each of the life history parameters. Multiple comparison was conducted using Least Significant Rank (LSR) to determine which groups were significantly different.

3. Results

Table 1 shows the life history parameters of amictic, unfertilized mictic and fertilized mictic females of *B. calyciflorus* fed on different concentrations of *S. obliquus*. The duration of juvenile period was lowest at mean food concentrations, whereas the durations of other periods tended to be highest at mean food concentrations. The duration of juvenile period was always the longest for fertilized mictic females. Among the food level-female type combi-
nations, the duration of juvenile period of unfertilized mictic female at the food concentration of 9.0 \times 10^6 \text{ cells mL}^{-1} was the longest. The reproductive period was longest for the unfertilized mictic females at low food concentrations, but at high concentrations the amictic females had longer reproductive times. Among the three types of females, the duration of reproductive period of amictic females and unfertilized mictic females was not significantly different, but both of them were significantly longer than that of fertilized mictic females. The post-reproductive period was (with the exception at 6.0 \times 10^6 \text{ cells mL}^{-1}) the shortest for amictic females. Apart from this concentration fertilized and unfertilized mictic females changed to have longer post-reproductive periods. The number of eggs was highest in mean concentrations. The number of the eggs of unfertilized mictic females was higher than that of fertilized mictic females. But the number of amictic eggs was always in between.

There were highly significant effects of both food level and female type on the duration of juvenile period, on the length of reproductive period and on the number of eggs produced per life cycle of the rotifer (Table 2). There were no significant effects of both food concentration and female type, independently and in interaction on the duration of post-reproductive period and the mean life-span of the rotifer. There was also an interaction between food level and female type in the duration of the juvenile period. This interaction was not found for all other developmental periods and the number of eggs.

The relationships between the duration of juvenile period, and the number of eggs of amictic females as well as fertilized mictic females and the food concentrations were all described by a curvilinear regression, respectively, but they differed in the sign of the parameters (Table 3). So at mean food concentrations the longest durations of juvenile periods were found but also the highest numbers of eggs.

### 4. Discussion

As far as the effect of food concentration on life history characteristics of amictic females of rotifers was concerned, prolonged juvenile periods at low food levels in *Euchlanis dilatata* (EHRB.) and *Brachionus rubens* (EHRB.) have been found by King (1967) and Pilar ska (1977). But in *Encentrum linnhei* (SCOTT) and *B. urceolaris* (ROUSSELET) there were no significant effects of food concentration on their juvenile periods (Schmid-Araya, 1991; Xi and Huang, 1999). The duration of reproductive period of amictic females in *B. rubens*, *B. urceolaris* and *Euchlanis dilatata* at low food concentrations were prolonged (King, 1967; Pilar ska, 1977; Xi and Huang, 1999). The lower and higher food levels made the lifespan of amictic females in *B. calyciflorus* and *B. urceolaris* shortened (Halbach and Halbach-
Contrary to those above, juvenile period of *B. plicatilis* was prolonged at lower and higher food concentrations. The effects of food level on the duration of reproductive period and the mean life-span of *Encentrum linnhei* and *B. plicatilis* were not significant (Schmid-Araya, 1991), which conformed with our results in this study.

The fecundity of amictic females of *B. calyciflorus*, *B. plicatilis*, *B. urceolaris* and *Encentrum linnhei* at higher and lower food levels was low (Halbach and Halbach-Keup, 1974; Schmid-Araya, 1991; Xi and Huang, 1999), and that of amictic females of *Asplanchna girodi* (Guerne) at lower food concentrations was also diminished (Roberson and Salt, 1981). In the present study, we found that in terms of egg numbers, the reproductive output of amictic females of *B. calyciflorus* was the largest at the food concentrations of 4.31 cells mL\(^{-1}\) (calculated from the equation), and at the lower and higher food levels it decreased, which agreed with the results obtained by Halbach and Halbach-Keup (1974), Schmid-Araya (1991) and Xi and Huang (1999).

At low food levels, the duration of juvenile and post-reproductive periods as well as the mean life-span of unfertilized mictic females of *B. calyciflorus* were prolonged (Galindo and Guisande, 1993), which disagreed with our results in this study. However, the effect of food concentration on the duration of reproductive period was not significant (Galindo and Guisande, 1993), which were similar to our results. It seems possible that the effect of food level on the duration of developmental stages varied with the tested range of food concentration, rotifer species and strains. In addition, we found that the effect of food level on the duration of juvenile period of fertilized mictic females of *B. calyciflorus* was also significant.

The relationship between the number of eggs produced by a fertilized mictic female and food level could be described by a curvilinear regression. But with respect to unfertilized mictic females, the relationship was not statistically significant, although the tendency that the mean value of egg number changed curvilinearly with the rise of food concentration occurred. Galindo and Guisande (1993) found that at limited food concentrations, the male net reproduction rate of mictic *B. calyciflorus* females was lowered by the available food, but when the food level was elevated, it increased until it reached an equilibrium with a mean of 22.45 males per mictic female. The reason for the discrepancy between their result and ours might be the difference in the tested range of food concentration.

*Asplanchna brightwelli* (Gosse) showed similar juvenile periods between amictic females and unfertilized mictic females (Pourriot and Rougier, 1991). Both the duration of reproductive period and the mean life-span of amictic females of *B. calyciflorus* were longer than those of unfertilized mictic females, but the duration of post-reproductive period was not significantly different in both types of females (Pourriot, 1973). In the present study, we found that the duration of the juvenile period among the three types of females was significantly different. Although the duration of the reproductive period between amictic females and unfertilized mictic females was not significantly different, both of them were longer than that of fertilized mictic females. The duration of post-reproductive period as well as the mean life-span was similar among the three types of females. The reason for the disparity between our results and those from above mentioned authors might be the difference in experimental condition, rotifer species or strains.

Among the three types of females, the relationship between the number of eggs produced by an amictic female as well as a fertilized mictic female and food level was statistically significant, but neither was that of an unfertilized mictic female. These might indicate that in terms of the number of eggs produced, the sensitivity to the change of food level among the three types of females was different. In addition, the number of eggs produced by an unfertilized mictic female was the largest, and that of a fertilized mictic female was the smallest, which was identical to the other two results of us (Xi and Huang, 2000a, b), but different from the results obtained by Pourriot (1973) and Dahril (1997). Pourriot showed
an identical net reproduction rate by amictic and unfertilized mictic females of *B. calyciflorus*. DAHRIL found that the number of eggs of an amictic female was larger than that of an unfertilized mictic female. The difference of number of eggs among the different types of females might be determined by physiological traits of the female and her eggs. Because the male egg was the smallest among the three types of eggs, the unfertilized mictic female had a low energy investment in a male egg (GILBERT, 1977) and could produce many eggs during her life cycle. In contrast, however, the resting egg possessed a bigger lipid reserve, the fertilized mictic female had a high energy investment in a resting egg and could just produce a few eggs during her life cycle. The unfertilized mictic females produce many males and are fertilized by them to ensure the population for resting egg formation. Conversely, amictic *B. calyciflorus* was capable of varying its reproductive pattern according to the food concentration to maintain a high rate of population growth (GUISANDE and MAZUELOS, 1991).

Both the duration of juvenile period and the number of eggs were significantly different among the three types of *B. calyciflorus* females. Amictic females of *Testudinella* (*Pterodina*) *elliptica* (EHRB.) were able to withstand osmotic pressure and pHs that caused reduced survival among mictic females (LUNTZ, 1926). The three types of *Euchlanis dilatata* females responded differently in net reproduction rate to temperature (KING, 1970). The asexual and sexual reproduction of *B. plicatilis* responded differently to temperature, salinity and food level (SNELL, 1986). The sensitivity of asexual and sexual reproduction of *B. calyciflorus* to four toxicants was different (SNELL and CARMONA, 1995). All those indicated that amictic and mictic females of rotifers possessed physiological diversity.

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6. References


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