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The Effect of Caloric Restriction on the Life Span and Reproduction of Fresh Water Rotifer (*Brachionus calyciflorus*)

Nilgun Ozdemir
Department of Aquaculture, Faculty of Agriculture, University of Ataturk, 25240 Erzurum, Turkey

Abstract: The effect of Caloric Restriction (CR) on life span of fresh water rotifer (*Brachionus calyciflorus*) was investigated in this study. Groups were fed at 12, 24, 36, 48, 60 and 72 h intervals with 0.5 mL (1.5×10^6 cell mL^{-1}) *Chlorella* sp. to apply CR. Significant changes were observed in maximum and average life spans in groups subjected to CR ($p<0.05$). Maximum life span in 36 h group was determined as 15 days, while it was 10 days in control group. Reproduction life span was observed to be increasing in spite of decreasing number of eggs. The results show that CR affected life span and reproduction in rotifer *B. calyciflorus*.

Key words: Rotifer (*Brachionus calyciflorus*), caloric restriction, dietary restriction, life span, reproduction

INTRODUCTION

Caloric Restriction (CR) is a type of low-calorie intake without causing malnutrition (Koubova and Guarente, 2006) and it may sometimes be defined as Dietary Restriction (DR) (Astagimath and Rao, 2004; Walker *et al*., 2005).

CR extends lifespan in a remarkable range of organisms from yeast to mammals (Weindruch and Walford, 1988; Masoro, 2000). Significant extension of life span under Caloric Restriction (CR) has been reported especially, for rotifer *Asplanchna brightwelli* (Verdone-Smith and Enesco, 1982) and *B. plicatilis* (Yoshinaga *et al*., 2000; Kaneko *et al*., 2005). Therefore, CR is one of the most widely used methods for understanding life span process today.

Because of their size, nutritional value and behavior, rotifers are the most important live food organisms used as starter for the rearing of fish larvae (Lubzens *et al*., 1989, 2001; Arimoro, 2006). The fresh water rotifer, *B. calyciflorus* is a suitable organisms and adequate food source for ornamental fresh water fish larvae such as Dwarf Gourami, *Colisa lalia* and Brown Discus (Sales and Janssens, 2003; Lima *et al*., 2003).

Hence, this study aims to contribute rotifer culture and sustainability of its culturing through the investigation of the effects of CR conditions on the life span of fresh water rotifer (*Brachionus calyciflorus*).

MATERIALS AND METHODS

Culture conditions: The rotifer used in this study was *B. calyciflorus* obtained from florida aquatic eco-systems Inc. (Apopka, FL) in the form of commercial eggs. The rotifers were fed with the single celled green algae *Chlorella* sp. Cultured in a Bold 1NV medium (Starr and Zeikus, 1993). For maintaining rotifer cultures hard water (EPA) was used as a medium (Hoff and Snell, 2001). Algae culture was consisted of a total of 3 glass beakers (1 L capacity) and cultured semi-continuously. Algal cell density was estimated using Neubauer haemocytometer under trinocular microscope (Novex WF 10×).

Cultures were conducted at 27±1°C, pH 7.0-7.5 and continous flourescent illumination (40 W 1000 lux). But experimental cultures of rotifer were kept under total darkness except during observations to prevent algal growth (Yoshinaga *et al*., 2000; Weithoff, 2007).

Experimental design: The CR was applied in method of lengthening the interval between the feeding range in rotifer groups by feeding them a monotype food (*Chlorella* sp.) containing same amount of cells (0.5 mL 1.5×10^6 cell mL^{-1}). Six groups of 4 rotifers were used for this study and the various groups were fed by replenishing their medium with fresh algae at intervals of 12, 24, 36, 48, 60, 72 h. Group that fed every 12 h was designated as the control group.

Numbered and lettered 24 well plates were used for determining the life span. Only 1 rotifer <6 h old was placed in each well and then they were subjected to CR conditions. Individual mothers were transferred to another well containing fresh EPA after reproduction using an automatic pipet in order to determine how many days they survive. Experiments were started in 4 replicates in each group and continued until all the individuals are dead (15 days).

The maximum Life Span (LS_{max}) and reproductive life span (LS_{rep}) of the rotifer were determined according to Kirk (2001).

Statistical analysis: Differences between groups were analyzed with one-way Analysis of Variance (ANOVA) and averages of the groups were tested using a multiple comparison test (Duncan) at theα = 0.05 level. The results are presented as means±SEM. The analyses were performed using SPSS version 13.0.

RESULTS AND DISCUSSION

Significant changes were observed in maximum and average life spans in groups subjected to CR. The maximum, mean life span and reproductive response for all the groups is given in Table 1. Figure 1 shows the survival curves for the 6 groups of *B. calyciflorus* fed at the various time intervals.

The mean life span of both 24 and 36 h groups were significantly longer than that of the control group that was 8.0±0.4 ($p<0.05$). In contrast, the life span 48 , 60 and 72 h groups were significantly shorter than of the control group ($p<0.05$). The life span of rotifers fed at 72 h intervals were shortest mean and maximum life span than of all groups. However, there was no significant differences in mean life span among 48 and 60 h intervals groups ($p>0.05$). Maximum life span remained unchanged in these groups (6 days), compared to the other groups.

It was observed during the experiments that individuals in all groups showed reproductive activity within the first 12 h after the feeding. Individuals were also observed to be stopping reproduction in the shortage of feed in the medium and extending reproduction periods for survival. The numbers of eggs for all the groups are given in Fig. 2.

As shown in Fig. 2, average number of offspring produced per rotifer was significantly reduced under severe CR.

In *Brachionus calyciflorus*, the results presented here show that underfeeding by means of extending the feeding interval 36 h increased the mean life span. It was also, observed that under CR conditions rotifer (*B. calyciflorus*) individuals preferred to survive by extending reproductive periods and that they resumed reproduction within the first 12 h after feeding. Moreover, the number of eggs was decreasing in accordance with the extended life span.

Although, rotifer have not got a very long life span under normal conditions, females can survive for 6-8 days and males can survive for approximately, 2 days under normal conditions (Hoff and Snell, 2001) and they stop reproduction (no >1 egg/individual) in parallel with decreasing feed levels and then resume reproduction

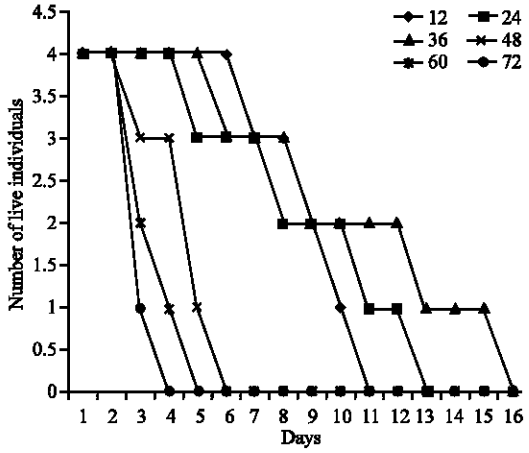

Fig. 1: Changes in life span under caloric restriction. Label numbers on graph shows the feeding intervals (h)

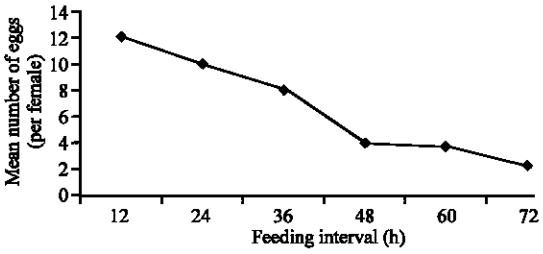

Fig. 2: Change in average number of eggs of *B. calyciflorus* after caloric restriction application

Table 1: Changes mean life spans and reproductive response of *B. calyciflorus* under CR conditions

Feeding interval (h)	Maximum life span (LS_{max})	Mean life span (days) ±SEM	Age of First Reproduction (AFR)	Average reproduction life span (LS_{rep})	Average No. eggs (per female)
12 (control)	10	8.0±0.400^{c}	1.0±0.000^{b}	7.25±0.85^{b}	12.0±0.910^{a}
24	12	11.5±0.290^{b}	1.5±0.290^{ab}	9.25±0.75^{ab}	10.0±0.410^{b}
36	15	14.0±0.410^{a}	1.75±0.25^{a}	11.0±0.800^{a}	8.0±0.410^{c}
48	6	6.0±0.410^{d}	1.75±0.00^{c}	4.0±0.410^{c}	4.0±0.410^{d}
60	6	6.0±0.410^{d}	2.0±0.250^{c}	4.0±0.410^{c}	3.75±0.25^{d}
72	5	4.75±0.48^{d}	2.5±0.280^{c}	2.0±0.000^{c}	2.25±0.25^{e}

$^{a-d}$Significant differences ($p<0.05$). Each value is the mean±SEM of 4 individual observations; SEM: Standard Error of Mean

within 3-5 days after the resuming of feeding (Ricci and Perletti, 2006). Yoshinaga *et al.* (2003a) reported that under normal and CR conditions the number of *B. plicatilis* offspring are 30 and 10, respectively and the life spans are observed to be approximately 10 and 30 days, respectively.

Enesco (1993) reported that long life span of rotifer under DR conditions is a result of the prolongation of reproductive period by the organisms themselves; however, there is no direct relationship between reproduction and longer life span. However, Yoshinaga *et al.* (2003b) suggested that the life span of rotifer *B. plicatilis* is shorter when, it is well fed and produces a large number of offspring. Thus, the relationship of trade-off between extension of life span and reproduction in rotifers under CR is still controversial and the mechanisms determining longevity have remained unknown (Kaneko *et al*., 2005).

In addition, as reported by Verdone-Smith and Enesco (1982), the average life span (4.41-4.35 days) of rotifer (*A. brightwelli*) fed in 48, 60 and 72 h intervals is shortened in comparison to rotifer fed in 12, 24 and 36 h intervals (5.44-6.08 days). The resulting values from especially control and 48, 60 and 72 h groups were similar to those of Verdone-Smith and Enesco (1982).

Hence, it is clearly shown in this study that CR significantly increases the life spans of rotifer *B. calyciflorus*. This situation observed under a CR is thought to be resulting from the self-limitation of reproduction activity. Yoshinaga *et al.* (2000, 2003a, b) argues that the prolongation of life span by the rotifer through self-limitation of reproduction is a response to resume reproduction in the future when normal food concentration conditions reconstituted.

On the other hand, according to Gilbert (2003), monogonont rotifers are quite flexible in allocating their energy between survival and reproductive activity under the scarcity of feed. For example, *B. plicatilis* may behave very flexibly in changing reproduction and life span. Moreover, it is found that when *B. plicatilis* reproduce in a dietary restricted, the offspring are more resistant to starve and such medium improve the quality of the offspring (Yoshinaga *et al.*, 2003b). As such, it is reported that since these species channelize all of their resources to reproduction in the absence of feed, their life span became shorter and accordingly, these species characteristically have short life span and try to survive by producing resting eggs through reproduction when nutritional conditions deteriorate (Ricci and Perletti, 2006). In contrast, Kirk *et al.* (1999) report that *B. calyciflorus* gives a physiological response to constrained nutritional medium by minimizing energy consumption through decreasing respiration rate, fecundity and egg size. It is also, argued in the same study that *S. pectinata* shows a totally opposite response than that of *B. calyciflorus*, thus, there are physiological differences among the different rotifer species under restricted diet. For example, in a study by Weithoff (2007) investigating the effects of DR on the life span and reproduction of 2 rotifer species (*Cephalodella* sp. ve *Elosa worallii*), only *E. worallii* survived longer (15 days) than the average life span (7 days). It is reported that during this time *E. worallii* extended its life span by slowing reproduction and started to reproduce again within 1 day after the normal nutritional medium provided. While, monogonont rotifer (*Synchaeta pectinata*) prefer to survive shorter in expense of reproduction during the state of starved, species such as *Keratella cochlearis* survive longer by delaying reproduction (Kirk, 1997). Yoshinaga *et al.* (2003b) found that young *B. plicatilis* that normally have a life span of 12 days survive >16 days during the state of starvation by slowing reproduction, however, the survival rate decrease during starvation due to aging.

Xi *et al.* (2001a, b) reported that food concentration and type have significant effects on the number of eggs produced by amictic *B. calyciflorus* females during their life cycle whereas the type of algae has no effect on reproduction period and life span. In contrast, Flores-Burgos *et al.* (2005) observed in their study of 2 rotifer species (*B. patulus*, *B. calyciflorus*) that algae type and concentration affected life span and reproduction rate. They argue that this difference in demographic parameters may be resulting from the specific ability of adaptation of these species to the changes in the composition of food. The effect of the type of algae is not researched in this study since, only one type of algae was used.

In our study, reproduction life span was observed to be increasing in spite of decreasing number of eggs. Thus, these results are in agreement with the results of such other studies as Kirk (1997, 2001), Yoshinaga *et al.* (2000, 2003b), Xi *et al.* (2001a, b) and Weithoff (2007).

CONCLUSION

It is clearly shown in this study that CR significantly increases the life spans of rotifer *B. calyciflorus*. However, rotifer's life span and reproductive response can vary due to culture conditions such as temperature, food type, food concentration and among the rotifer species.

On the other hand, more severe restriction was not useful to of the *B. calyciflorus* culture because of high

concentration of food is being given in order to produce dense amount of rotifers in a short time in culture. But high concentration of food can cause the deformation of the culture water and even cause the collapse of the entire culture in short time. Whereas, it can possible to obtain normal population increase with small amount of food (such as feeding between the 24 and 36 h interval). It can also, being consider that restricted diet may decrease feeding costs in the rotifer cultures and extend life span of these organisms to achieve sustainability of the culture.

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