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Optimization of Indoor Production of Fresh Water Rotifer (*Brachionus calyciflorus*): A Preliminary Study

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ABSTRACT

Recently rotifer culture projects were undertaken at selected places in Punjab province. Rotifers are fed on *Chlorella vulgaris ad libitum* and exact algal requirements are not cared for which increases the production cost of rotifers. Therefore in the current studies, algal cell requirement of *Brachionus calyciflorus* was determined for its cost effective production and utilization of data acquired in similar studies or in commercial applications. Required number of rotifers were withdrawn from the main stock, divided into three groups and fed on known number of laboratory grown *C. vulgaris.* Left over cells were counted after 24 h in all the three groups and their ingestion and filtration rates were determined. Algal cell requirements of *B. calyciflorus* were therefore determined which came out 32977 to 35540 cells/rotifer/day. In the second trial rotifers were cultured in cow-dung media with or without supplemental aeration. Jars aerated for 24 h produced highest rotifer density (350/mL), while those with 12 h aeration ranked second and those without aeration even could not support hatching of cysts. In the third trial effect of type and size of container on rotifer production was determined. Significantly higher rotifer density was observed in jars (380 rotifers/mL) compared with flasks (174/mL). The fourth trial was meant to determine the effect of culture duration on rotifer density. Highest number (135 rotifers/mL) was observed after 15 days of inoculation, the rotifers then started to decline and were 115/mL after 26 days of inoculation.

Key Words: *Brachionus calyciflorus*; Ingestion rate; Supplemental aeration

INTRODUCTION

In current fish culture practices larviculture is one of the main bottlenecks in the promotion of the production of fish and crustaceans. The larvae of culturable Indian and Chinese major carps hatch in relatively undeveloped state. They depend on the nutrients stored in the yolk sac for the first few days. On completion of yolk sac absorption, they demand immediate external food for nourishment. They respond best to motile prey organisms, which are important food as well as enzyme source to digest food (Chew & Lim, 2005/2006). Hence culture of live food is an important component of a successful fish hatchery (Lee *et al*., 2002). Non-availability of appropriate food at this stage is a major cause of larval losses.

Rotifers have been widely used as essential food source in raising freshwater and marine fish larvae due to its unique characteristics (Lubzens, 1987; Dhert, 1996). It is easily digestible, has appropriate size, can survive in high stocking densities and swims slowly giving an ample opportunity to its predator for prey (Qie *et al*., 1997; Lubzens *et al*., 2001). It possesses apposite biochemical composition that suits the nutritional requirements of larval fish. Moreover, it has the potential for enrichment with fatty acids and vitamins and various therapeutants for production of healthy fish (Maragelman *et al*., 1985). Therefore successful culture of fish and shrimp in various parts of the world can be attributed partly if not totally to successful mass cultivation of rotifers.

With the remarkable developments in larval rearing technology of important food fishes, demand for rotifers has increased considerably. Accordingly research has been focused on high stocking density culture, identification of appropriate food species and control of bio-chemical factors, which hinder the mass production of rotifers. Not much attention has been given on its culture in freshwater in general and in our local environment in specific. We need this minuscule in abundance to make our fish hatcheries a successful venture. Therefore in the present studies some aspects for mass production of this minuscule viz.

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determination of its algal cell requirements, effect of supplemental aeration, duration of culture and type and size of container on rotifer production were investigated.

MATERIALS AND METHODS

Experimental site: The studies were conducted simultaneously at two different places; Fish Hatchery Faisalabad and Fisheries Research and Training Institute, Lahore and were based on freshwater rotifer, *Brachionus calyciflorus*.

Preparation of stock culture of rotifers: Mixed population of copepods, rotifers and cladocerans, was collected from the wild. The water containing rotifers was sifted through 600, 212, 125, 75 and 38 µm sieves arranged one above the other in a decreasing downward order. The material collected in the bottom most screen, was observed under microscope for confirmation of desired fauna, which was saved for future rotifer culture. Repeated isolations using a Pasteur pipette and transfers were achieved under light microscope at 10X magnification.

Starter culture: The starter culture consisted of a static system and was limited to 500 mL Erlenmeyer's flasks. The inoculated flasks were placed at 2 cm from fluorescent light tubes (500 lux). The temperature was approximately constant at 28°C. Sufficient aeration was provided through the perforated stones immersed in water and based at the bottom of the flask. The rotifers were stocked at the density of 30/mL and fed with freshly harvested *Chlorella* algae containing 1.6×10^6 cells/mL. Successful starter culture was maximized to meet the requirement of different trials. The detail of these trials has been given in the subsequent paragraphs under this section.

Algal requirement of rotifers: These were the fundamental studies and base of all the succeeding trials. The rotifers were housed in 6 L fiberglass jars. Each jar containing 5 L water was inoculated with 150 rotifers. There were three treatments and a control (Table I). Each treatment including control had three replicates. The experimental units were randomly allotted to different treatments. The rotifers in three groups were provided with varying quantities of algae, while the fourth one which served as control, was starved.

Count of algal cells and determination of ingestion rate of rotifers: Neubauer haemocytometer was well cleaned and used for algal cells (Bauer, 1990). Ruled areas were centrally covered with cover slip. A well mixed algal sample was placed in the "V" groove of the metal surface. Both chambers were gradually filled to eliminate air bubbles and for even distribution of algal cells throughout the counting chamber. Counting was started from the top left square and only those cells were counted, which lay within or touching the boundary line of the square. All the cells counted in individual block were added up and cell density was calculated by the mathematical expression below:

$$D \text{ (cells/mL)} = (\text{Total count} \times 10^4 / \text{No. of blocks})$$

Filtration and ingestion rates of rotifer were calculated following Yufera and Pascual (1985) (Table I):

$$\text{Filtration rate } F = (\text{Ln } C_0 - \text{Ln} C_t)/V.t$$

$$\text{Ingestion rate } IR = F. \sqrt{C_0.C_t}.$$

Where C_0 and C_t are initial and final cell densities, t = time and V = zooplankton density.

Effect of supplemental aeration: Trial was conducted in jars. The jars in each trial were randomized to minimize errors due to external factors. Group 1 was kept as control (without aeration) and other two groups were provided with appropriate aeration source to maintain a desired level of oxygen. Each group including control was triplicated. Inoculant and ration size was same as used in above trial. The studies were continued for 30 days. Random samples of rotifers were taken from each jar. Rotifers were counted and number per mL was recorded (Table II).

Effect of experimental duration: Pursuing above mentioned feeding regimes, rotifers were reared in three 6 L jars up to 26 days. Their density was estimated at 10, 15 and 26 day of inoculation in each container to determine concentration of rotifers. Rotifers observed were counted and recorded (Table III).

Effect of type and size of container: During this trial rotifers were cultured in 500 mL glass flasks and fiberglass jars. All the treatment groups were triplicated. Both the groups were fed on Chlorella@approximately 35000 cells/rotifer. Level of feeding and aeration was same in all the three containers of each treatment. Rotifers were reared for 26 days. The intrinsic rate of natural increase, doubling time in days and population growth rate were calculated following James and Dias (1984) and Hamada *et al*. (1993) ((Table IV) (Fig. 1a, b & Fig. 2a, b):

$$\text{Intrinsic rate of natural increase} = r = \text{Ln}(N_t) - \text{Ln}(N_0)/t$$

$$\text{Doubling time of population in days} = tD = 0.6931/r$$

$N_t = N_0 - e^{rt}$ where N_t and N_0 = final and initial populations,

$$\text{Generation time} = \text{Gent't} = 1 \div \text{Divisions day}^{-1}$$

$$\text{Divisions day}^{-1} = \text{Div. day}^{-1} = K \div \text{Ln}2.$$

RESULTS

Estimation of algal cell requirements of rotifers: All the algal cells were consumed in treatment 3 (25600 cells/rotifer) while approximately 33000 (59000 rotifer/jar) and 35000 (75800 rotifer/jar) cells were consumed in treatment 2 and 1 respectively in 24 h time period (Table I). Ingestion and filtration rates were 35540 and 0.0043, 32977 and 0.0057, 198.05 and 0.10107 in treatment group 1, 2 and 3, respectively. There were no differences in group 1 and 2 but both these groups were significantly different ($p<0.05$) from group 3 (Table I).

Effect of supplemental aeration: Significantly ($p<0.05$) higher number of rotifers (350/mL) were observed in 24 h aeration group than the group where aeration was

Table I: Estimation of algal consumption by *B. calyciflorus*. Each jar contained 5 L water, there were 30 rotifers L^{-1} and 150 rotifers jar^{-1}

Treatment	No. of rotifers inoculated jar^{-1}	No. of algal cells introduced $rotifer^{-1}$	Total No. of algal cells jar^{-1} (×1000)	Total No. of algal cells mL^{-1} (×1000)	Total No. of cells recovered after 24 h mL^{-1} (×1000)	Total No. of cells recovered after 24 h jar^{-1} (×1000)	No. of algal cells consumed mL^{-1} (×1000)	Total No. of cells consumed in 24 h jar^{-1} (×1000)	Total No. of rotifers recovered jar^{-1}	Filtration Rate (FR)	Total No. of algal cells consumed $rotifer^{-1}$ rate (×1000)
4(Control)	150±3.1	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Cysts only	Nil	Nil
1	150±3.4	75.8±2.30	11370±806	2.27± 0.20	1.19 ± 0.16^{a}	5945 ± 350^{a}	1085 ± 198^{a}	5425 ± 3.47^{a}	151 ± 3.6^{a}	0.004323	35.54 ± 3.46^{a}
2	150±2.3	59.0±1.83	8850± 790	1.77± 0.19	0.75 ± 0.10^{b}	3750 ± 298^{b}	1020 ± 201^{a}	5100 ± 3.28^{a}	153 ± 2.5^{a}	0.005724	32.98 ± 2.99^{a}
3	150±3.1	25.6±1.56	3840± 456	0.77±0.11	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	760 ± 156^{b}	3840 ± 2.68^{b}	142 ± 3.2^{a}	0.10107	19.81 ± 2.38^{b}

Note: Figures with different superscript letters are significantly different from each other at $p<0.05$.

Table II: Rotifer culture in 6 liter fiber glass jars with supplemental aeration, Cow dung was added every other day @ 0.5 kg per jar and studies were continued for two weeks.

	Rotifer Production Data			Water Quality Data				
Inputs	Quantity used (kg)	Level of aeration	# rotifers mL^{-1}	pH	Alkalinity (ppm)	DO (ppm)	Hardness (ppm)	TDS (ppm)
Cow dung extract	0.5 kg	Nil	Resting eggs only	7.25	305	Not detected	510	4000
-do-	0.5	12 hour	102 ± 6.1^{a}	7.15	295	3.9	550	4100
-do-	0.5	24 hour	350 ± 30.2^{b}	7.25	305	7.2	540	4200

Table III: Effect of experimental duration on production of rotifers, Total water volume was 5 L and there were 30 rotifers mL^{-1}, Green water was 1L and algal cells were 2.64×10^{6} mL^{-1} (total cells $L^{-1} = 2.64 \times 10^{6}$ cells $ml^{-1} \times 1000$ mL)

	Rotifer Production Data			Water quality data			
Treatment	No of days after inoculation	No. of rotifers observed mL^{-1}	pH	Alkalinity (mg L^{-1})	DO (mL L^{-1})	Hardness (mg L^{-1})	TDS (mg L^{-1})
10	10	105 ± 10.5^{a}	7.8	310	4.0	520	4100
15	15	135 ± 12.2^{b}	8.1	300	3.9	530	4150
20	20	120 ± 12.4^{a}	8.3	305	4.3	540	4170
26	26	115 ± 11.0^{a}	8.0	299	4.6	525	4099

Table IV: Effect of type of container on rotifer production

Parameters	Flasks		Jars	
Mean growth rate	0.0147±0.0359	1.47%	0.0284±0.036	2.84%
Median growth rate	0.022	2.2%	0.043	4.3%
Maximum growth rate	0.589	58.9%	0.492	49.2%
Maximum density (mL^{-1})	174		380	
Divisions day^{-1}	0.0212		0.0409	
Generation time (days)	47.094		24.421	
Generation time (h)	1130.26		586.10	

restricted to 12 h (102/mL). There was no rotifer in non-aerated group and only cysts were observed in jars (Table II).

Effect of experimental duration: Maximum number of rotifers was observed on day 15 of inoculation. Rotifers density then gradually declined and there were only 115 rotifers on day 26 (Table III).

Effect of type of container: Rotifers were cultured in 1 L flasks and 6 L fiber glass jars. Peak rotifer density was observed in flasks on day 9, while in jars on day 11. When the total number of rotifers produced were compared for both treatments, the number was significantly ($p<0.05$) higher in jars than flasks. Mean growth rate of rotifers and doubling time was 1.47% and 2.84%, 47 days and 24 days in flasks and jars, respectively (Table IV) (Fig. 1a, b & 2a, b).

DISCUSSION

Determination of algal cell requirements of rotifer is the basis for investigative studies on any aspect of rotifer culture. This study showed that each rotifer consumed 35540, 33000 and 25600 cells in treatment 1, 2 and 3, respectively (Table I). Group 1 and 2 consumed significantly higher ($p<0.05$) algal cells than group 3, where number of total cells provided were also far lesser than group 1 and 2. Like other treatments consumption rate in group 3 might have increased if number of cells in the container would have been equaled to former treatments. Nevertheless this gives a reasonable estimation of daily algal cell requirement of individual rotifer which is approximately 33000 to 35000 algal cells/day.

Previously Hirata (1989) has reported 16789 *Nannochloropsis* cells per marine rotifer, *B. plicatilis*. Lavens and Sorgeloos (1996) found that decrease in cell density did not decrease the rotifer number up to day 3 of inoculation, while Chew and Lim (2005/2006) noted that with decreased daily feeding, the number of rotifers increase over the duration of the experiment. Similarly, Ajah (2008) observed continuous increase in the population of *A. priodonta* upon decrease in algal population. On the other hand Bently *et al.* (2008) has noted much higher number of non-viable algal cells (68000) per rotifer indicating that not only environmental conditions but the nature of algae also

Fig. 1: Rotifer production trend (a) in 500 mL flasks and their intrinsic growth rate (b)

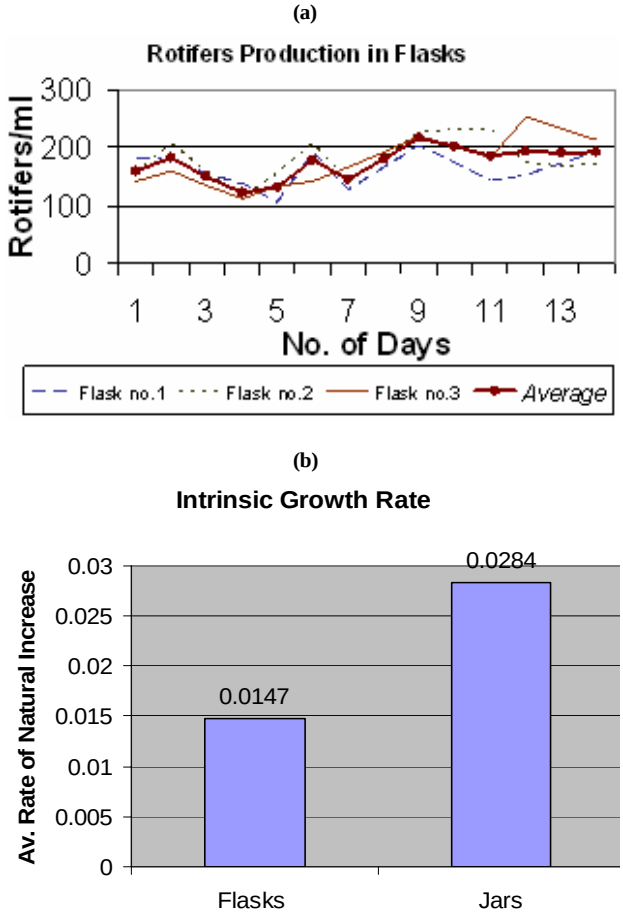
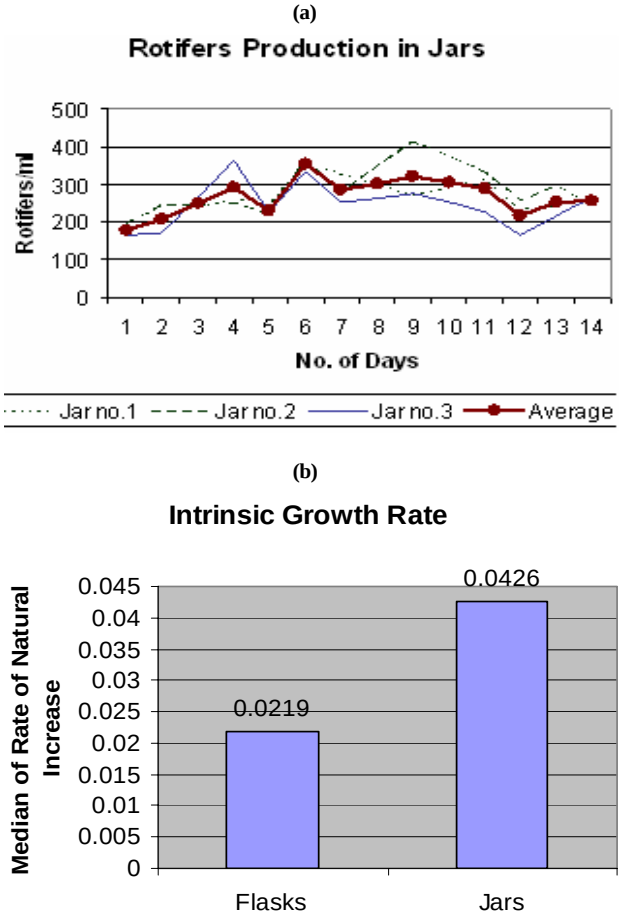


Fig. 2: Rotifer production trend (a) in 6 L jars and their intrinsic growth Rate (b)



affects requirement.

The estimate of rotifers can be translated into ingestion (IR) and filtration rates (F) of rotifers, which could be summarized as acceptability and catch efficiency of the cell suspension. They are appropriate measurements and are closely related to feeding behavior. In current studies there was increase in ingestion or intake rates with the rise in algal density, while there was proportionate decrease in filtration rates from treatment 1 to 3 (Table I). The IR and F are influenced by several intrinsic cell factors such as size, shape, chemical composition, physiological state and concentration of cells (Starkweather, 1980). Bogdan and Gilbert (1982) have reported positive correlation ($p<0.05$) between F and IR in all three algae compared. Higher F and IR occurred in their studies at decreasing food densities up to a certain concentration and always zero in all three algae examined whenever cell concentration was lower than the initial concentration. Contradictory to these studies filtration rate always increased in our studies with the decrease in the number of cells in the culture container (Table I). Pourriot (1977) and Starkweather (1980) showed increased IR with food density at lower concentration and constant at higher algal densities. May be lower food concentration increases the competition among the organisms, which accelerates their filtration rate. But this is not always true and exceptions are there (e.g., Yufera & Pascual, 1985). This study partially contradicts our observations but major part favorably agrees with ours further supporting and confirming the validity of our findings.

Effect of experimental aeration was observed on rotifers in cow dung media. Twenty four hour aeration produced 350, 12 hour 102 and only cysts were observed without supplemental aeration (Table II). All the groups were significantly different from each other indicating a pivotal role of aeration in rotifer production. Treece and Davis (2000) investigated the role of feed and extensive aeration on the production of rotifers. They observed that normal concentration of rotifers is 100-200 individuals/mL but often reaches 1000/mL with an adequate food and air supply. In line with our observations, Dahril *et al*. (1998) did not observe any appreciable growth of rotifers in animal manure and all the rotifers died on day 4 of inoculation. The existence of bacteria in the culture medium did not support the population growth of *B. calyciflorus*. Chew and Lim (2005/2006) further confirmed that aeration, which determines the DO, is a critical factor in the application of

inert diets. He further stressed that DO must be monitored to ensure adequate DO level (not <3 ppm) and sufficient turbulence to allow feed and rotifers in suspension. Though we could not get high rotifer density in our experiment as the former researchers got but we did prove the pivotal role of aeration in culture of rotifers and further warrant that rotifer production is difficult without aeration no matter how good the culture media are.

The experimental duration revealed a peak of production of rotifers on day 15 of inoculation, beyond that, the rotifer number started to decline and lowest was on day 26 (Table III). This is not the normal behavior of rotifers growth, since Lavens and Sorgeloos (1996), Chew and Lim (2005/2006) observed peak density of rotifers on day 3 and day 4, respectively. The appearance of peak during current studies may be due to several physico-chemical and biological factors, which need further deliberations and experimentation, which will be of our future investigative work. Nevertheless, Rhodes (2003) in his studies give some clue of this irregular rotifer behavior and states that after day 21, the proportions of the various life stages in the population become more uniform and the effect of the length of the treatment on 'r' may diminish after the stable stage population has been reached, if conditions remain favorable for growth. This too does not seem too relevant to the abnormal behavior observed during current studies and do not fully justify this indiscretion.

When rotifer production was compared in flasks and jars, higher growth rates, densities, divisions per day and lower generation time was observed in jars, while the vice versa was true in flasks (Table IV). Rhodes (2003) compared the type and size of containers in the production of *Nitokra lacustris*, a marine harpacticoid copepod and found that trays had significantly ($p<0.05$) higher population densities and growth rates ($p<0.05$) than carboys. We have the similar findings too (Table IV; Fig. 1 & 2). Previous (Rhodes, 2003) and our studies reveal that open tanks have more air to water surface contact, which in turn facilitated more rotifer production indicating that not only internal aeration but external diffusion also matters. Surface area in this study refers to the air-water interface instead of the total surface of the container available to the rotifers. It appears that total container surface area to volume ratio may be more relevant to population growth (StØttrup, 2000). This may depend on the behavior of the rotifers. Rotifers grown in poor tank hygiene can get more benefit from a larger surface area to volume ratio, which has been shown to be important in the culture of other harpacticoid copepods (Heath, 1994). Comprehensive studies of these and some other factors will be focus of our future research plan.

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