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Influence of salinity on population growth and reproductive performance of the rotifer, *Brachionus plicatilis*

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Abstract

Rotifers are important live feed organisms in aquaculture due to their high reproductive capacity, small size and suitability for feeding marine fish and crustacean larvae. The present study was conducted to determine the effect of different salinity levels on population density and growth rate of *Brachionus plicatilis* under laboratory conditions. The experiment was performed at the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval for 10 days using five salinity treatments (10, 15, 20, 25 and 30 ppt) with three replications. Rotifers were stocked at 10 individuals mL⁻¹ and fed with *Nannochloropsis oculata*. Significant differences were observed among treatments. The highest population density and growth rate were recorded at 15 ppt salinity, followed by 20 ppt, while the lowest performance was observed at 30 ppt. The study concludes that moderate salinity levels (15-20 ppt) are favourable for enhancing population growth and efficient mass culture of *B. plicatilis* as live feed for aquaculture systems.

Keywords: *Brachionus plicatilis*, rotifer culture, salinity, population growth, live feed, aquaculture

Introduction

Rotifers are widely used as live feed organisms in commercial fish and crustacean hatcheries throughout the world. Their suitability as a culture organism is mainly due to their planktonic nature, small size, slow swimming ability, high reproductive rate, tolerance to a wide range of environmental conditions and ability to be cultured at high densities. Several rotifer species such as *Brachionus plicatilis*, *Brachionus rotundiformis*, *Brachionus calyciflorus*, *Brachionus angularis*, *Brachionus rubens* and *Brachionus patulus* are commonly used as live food for marine, brackishwater and freshwater fish larvae and crustacean culture (Anitha *et al.*, 2015) [5].

Among these species, *Brachionus rotundiformis* is widely preferred in marine larviculture due to its smaller body size, which makes it suitable for feeding fish larvae with small mouth openings. The ability of rotifers to tolerate different salinity conditions makes them highly valuable for aquaculture production. *Brachionus* species can survive over a wide salinity range, although optimum growth is generally observed at lower salinity levels below 35 ppt (Lavens & Sorgeloos, 1996) [13].

Salinity is one of the most important environmental factors influencing the biological characteristics and population dynamics of rotifers. It affects important physiological processes including osmoregulation, reproduction, growth and survival. Rotifers maintain ionic balance between their body fluids and surrounding environment, and changes in salinity influence energy allocation between reproduction and survival. Previous studies have shown that salinity significantly affects fecundity, developmental time and lifespan of *Brachionus* species (Snell, 1986; Miracle & Serra, 1989) [19, 17].

The salinity requirement of *B. rotundiformis* varies among strains and geographical populations. Different studies have reported optimum salinity ranges from 11 ppt to 25 ppt for better growth and reproduction (Hagiwara *et al.*, 1995) [11]. Therefore, understanding the effect of salinity on population growth is essential for optimizing mass culture techniques and ensuring a continuous supply of high-quality live feed for aquaculture systems.

Materials and Methods

The experiment was conducted at the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval. Rotifer samples were collected during April and May from three coastal locations of Veraval, Gujarat: Triveni Sangam (20.884°N, 70.412°E), Devka River (20.927°N, 70.353°E) and Jaleshwer (20.9169°N, 70.344896°E) using fine plankton nets of 45 µm and 60 µm mesh size (Thomas *et al.*, 2016) [20]. The collected samples were examined under a compound microscope for species identification and preserved in 4% formalin (Anitha & George, 2006) [3]. *Brachionus plicatilis* was selected and stock cultures were maintained under laboratory conditions following standard rotifer culture techniques (Lubzens *et al.*, 2001) [15]. Rotifers were fed with *Nannochloropsis oculata*, and algal density was maintained using a Neubauer haemocytometer (Brown *et al.*, 1993; Andersen, 2005) [7, 1]. Seawater used for culture was pretreated by mechanical filtration and chlorination to remove unwanted microorganisms and contaminants. Filtered seawater was disinfected with sodium hypochlorite and residual chlorine was neutralized using sodium thiosulphate before use (Creswell, 2010; Madhu, 2009) [9, 16]. Five salinity treatments were prepared using treated seawater diluted with distilled water as S₁ (10 ppt), S₂ (15 ppt), S₃ (20 ppt), S₄ (25 ppt) and S₅ (30 ppt) to evaluate the effect of different salinity levels on rotifer population growth. The required salinity levels were prepared using the formula $V_1S_1 = V_2S_2$ (Boyd, 1998) [6]. The experiment was conducted in a Completely Randomized Design (CRD) with five salinity treatments and three replications. Rotifers were stocked at an initial density of 10 individuals mL⁻¹ and cultured for 10

days. Daily sampling was carried out, and population density was estimated using a Sedgewick-Rafter counting chamber. Specific population growth rate was calculated according to Hoff and Snell (1987). The obtained data were analysed using two-way ANOVA under CRD, and treatment differences were tested following Snedecor and Cochran (2014) [18].

Results

Effect of Salinity on Population Density of *Brachionus plicatilis*

The population density of *Brachionus plicatilis* showed a gradual increase from day 1 to day 10 under all salinity treatments (S₁-S₅). The overall mean population density varied significantly among the treatments. The highest population density was recorded in S₂ (15 ppt) (425.36 nos. mL⁻¹) followed by S₃ (20 ppt) (387.94 nos. mL⁻¹), S₁ (10 ppt) (316.04 nos. mL⁻¹), S₄ (25 ppt) (260.05 nos. mL⁻¹) and the lowest population density was observed in S₅ (30 ppt) (196.04 nos. mL⁻¹). Statistical analysis showed significant differences ($p < 0.05$) among salinity treatments and salinity × culture period interaction.

During the experimental period, population density increased continuously and reached maximum values on the 9th day. The highest density was observed in S₂ (15 ppt) with 722.08±448.17 nos. mL⁻¹, followed by S₃ (20 ppt) with 661.29±409.44 nos. mL⁻¹ on day 9. The lowest density was recorded in S₅ (30 ppt) throughout the culture period. These results indicate that moderate salinity levels (15-20 ppt) were more suitable for population multiplication of *B. plicatilis*.

Table 1: Influence of temperature (°C) and culture period (days) on the population growth (nos./mL) of the rotifer (*Brachionus plicatilis*) (Mean ± SD; n = 3)

TIME	T ₀	T ₁	T ₂	T ₃	MEAN
DAY 1	12.93±1.59	14.1±1.50	13.5±1.29	10.76±0.37	12.8
DAY 2	35.16±9.70	53.13±14.8	58.86±16.7	11.63±1.48	39.7
DAY 3	82.66±24.62	129.03±42.56	144.8±43.4	14.76±2.51	92.8
DAY 4	160.66±49.28	257.43±82.96	292.73±89.0	19.46±3.43	182.5
DAY 5	268.26±82.27	414.73±119.97	506.33±152.6	23.63±4.65	303.2
DAY 6	397.03±118.13	584.33±161.74	657.33±188.1	27.26±6.60	416.4
DAY 7	509.53±145.86	728.5±195.89	735.93±199.98	29±7.80	500.7
DAY 8	585.1±162.53	845.93±223.35	669.86±186.7	25.26±8.64	531.5
DAY 9	631.2±167.42	921.73±234.97	611.36±173.26	21.73±8.44	546.5
DAY 10	648.03±169.44	955.1±238.27	555.86±160.05	18.7±7.73	544.4
TOTAL (MEAN)	333.06 ^c	490.40 ^a	424.66 ^b	20.22 ^d	
Treatment	S.Em.±	C.D. at 5%	C.V.%		
D (Days)	1.462	4.06	6.12%		
T (TEMPERATURE)	154.733	2.56			
T × D	2.923	8.12			
TWO-WAY ANOVA TABLE					
Source of variation	Sum of squares	degree of freedom	Mean sum of squares	F cal	F tab 5%
D	25921147	9	2880127	22475.75	2.01
T (Temperature)	19499051	3	6499684	50721.82	2.6
T x D	10770327	27	398901	3112.918	1.62
Error	51257.5	400	128.144		-
Total	64277807	599		-	-

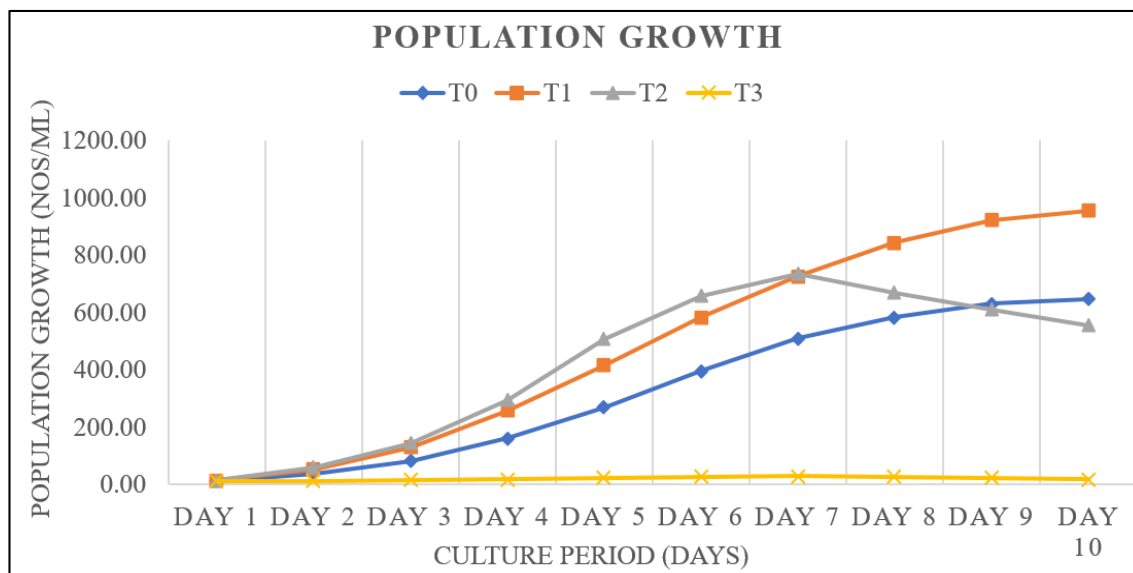


Fig 1: Influence of temperature (°C) and culture period (days) on the population growth (nos./mL) of the rotifer (*Brachionus plicatilis*)

Effect of Salinity on Population Growth Rate of *Brachionus plicatilis*

The specific population growth rate of *Brachionus plicatilis* varied significantly under different salinity treatments. The highest growth rate was recorded in S₂ (15 ppt) (0.34 day⁻¹) followed by S₃ (20 ppt) (0.32 day⁻¹), while the lowest growth rate was observed in S₅ (30 ppt) (0.21 day⁻¹). The treatments S₁ (10 ppt) and S₄ (25 ppt) showed intermediate

growth rates. Statistical analysis showed significant differences ($p < 0.05$) among salinity treatments.

During the culture period, the growth rate increased up to the peak growth phase and declined slightly towards the end of the experiment. The maximum population growth rate in 15 ppt salinity indicated that this salinity level was more suitable for reproduction and multiplication

Table 2: Influence of temperature (°C) and culture period (days) on the growth rate (r) of the rotifer (*Brachionus plicatilis*) (Mean $\pm$ SD; n = 3)

TIME	T ₀	T ₁	T ₂	T ₃	MEAN
DAY 1	0.2504±0.11	0.3382±0.108	0.2959±0.094	0.0733±0.033	0.2394
DAY 2	0.9707±0.17	1.2921±0.221	1.4387±0.204	0.0709±0.134	0.9431
DAY 3	0.8487±0.03	0.8717±0.124	0.8959±0.040	0.2325±0.071	0.7122
DAY 4	0.6617±0.023	0.6956±0.133	0.7024±0.017	0.2753±0.019	0.5838
DAY 5	0.5125±0.014	0.4851±0.072	0.5470±0.042	0.1906±0.022	0.4338
DAY 6	0.3943±0.019	0.3465±0.020	0.2646±0.022	0.1319±0.108	0.284
DAY 7	0.2521±0.018	0.2221±0.019	0.1169±0.017	0.0527±0.056	0.1610
DAY 8	0.1400±0.017	0.1506±0.018	-0.0965±0.011	-0.1698±0.144	0.0061
DAY 9	0.0788±0.017	0.0880±0.015	-0.0930±0.007	-0.1804±0.109	-0.0266
DAY 10	0.0270±0.011	0.0370±0.0024	-0.0971±0.011	-0.1683±0.073	0.0504
TOTAL (MEAN)	0.4136 ^b	0.4526 ^a	0.3974 ^c	0.0508 ^d	
Treatment		S.Em.±	C.D. at 5%		C.V.%
D (Days)		0.006	0.01		6.12%
T (TEMPERATURE)		0.023	0.06		
T × D		0.012	0.03		
TWO-WAY ANOVA TABLE					
Source of variation	Sum of squares	degree of freedom	Mean sum of squares	F cal	F tab 5%
D	25921147	9	2880127	22475.75	2.01
T (Temperature)		19499051		3	6499684
T × D		10770327		27	398901
Error		51257.5		400	128.144
Total		64277807		599	-

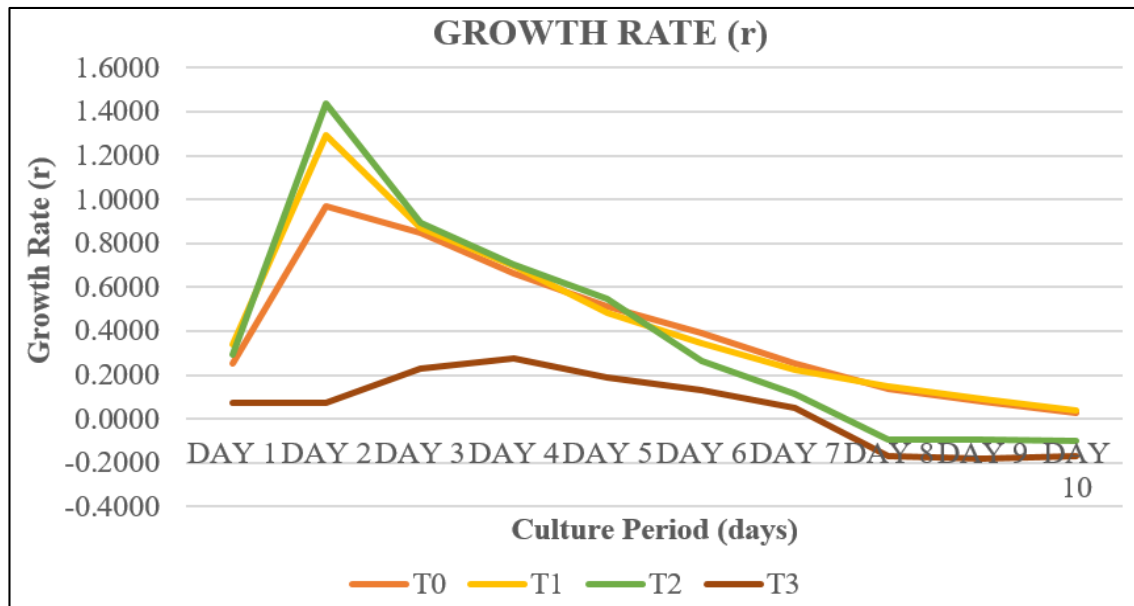


Fig 2: Influence of temperature (°C) and culture period (days) on the growth rate (r) of the rotifer (*Brachionus plicatilis*)

Discussion

Effect of Salinity on Population Density of *Brachionus plicatilis*

In the present study, salinity significantly influenced the population growth of *Brachionus plicatilis*. The highest population density was observed at 15 ppt salinity, followed by 20 ppt, indicating that moderate salinity conditions provided favourable environmental conditions for rotifer reproduction and growth. Similar findings were reported by Cheng *et al.* (2011), who observed maximum population growth of *Brachionus rotundiformis* at 10 and 20 ppt salinity.

The better performance at moderate salinity may be due to reduced energy requirements for osmoregulation, allowing more energy to be utilized for reproduction and population increase. Gelbic *et al.* (2000) reported maximum population density of *B. plicatilis* at 20 ppt salinity, which supports the present observation where higher population density was recorded at 15 and 20 ppt treatments.

The lower population density at higher salinity levels (25 and 30 ppt) may be attributed to increased osmotic stress, which affects feeding activity, reproduction and physiological balance of rotifers. Similar results were reported by Lee *et al.* (2022) [14], who observed reduced reproductive performance of *Brachionus rotundiformis* at higher salinity levels. Therefore, maintaining optimum salinity is important for successful mass culture of *B. plicatilis* in aquaculture systems.

Effect of Salinity on Population Growth Rate of *Brachionus plicatilis*

The present study showed that salinity significantly affected the specific population growth rate of *Brachionus plicatilis*. The maximum growth rate was observed at 15 ppt salinity, followed by 20 ppt, indicating that moderate salinity conditions were favourable for rotifer reproduction and population increase. Similar results were reported by Snell (1986) [19] and Miracle and Serra (1989) [17], who observed that salinity influences reproductive activity and growth rate of *Brachionus* species.

The higher growth rate at moderate salinity may be due to lower energy requirements for osmoregulation, allowing

more energy to be utilized for feeding, reproduction and population development. At higher salinities (25-30 ppt), the decrease in growth rate may be related to osmotic stress, which affects physiological activities and reduces reproductive efficiency. Lubzens *et al.* (2001) [15] also reported that optimum salinity conditions are essential for maintaining high rotifer productivity in aquaculture systems. The findings suggest that 15-20 ppt salinity is suitable for improving population growth and mass culture of *B. plicatilis* as a live feed organism for aquaculture.

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