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Effects of salinity, temperature, feed type and feed concentration on the reproductive rate of *Brachionus angularis*

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Abstract

The impact of salinity, temperature, feed type and feed concentration on reproductive rate 'r' of *Brachionus angularis* were examined in the present study. Three salinity levels (0.5%, 5.0% and 10.0%), three temperature regimes (25±1 °C, 29±1 °C and 35±1 °C) and four microalgal diets, *Chlorella ellipsoidea*, *Ankistrodesmus convolutes*, *Scenedesmus protuberans* and *Chlorococcum infusorium* at a concentration of 1x 10⁶, 2x 10⁶, 3x 10⁶, 4x 10⁶, 6x 10⁶, 8x 10⁶, 10x 10⁶ and 12x 10⁶ were tested in the experiment. The findings showed that all these parameters have significant influence on reproductive potential of *B. angularis*. The optimum salinity and temperature required for highest 'r' were 0.5% and 28.0 °C, respectively. The intrinsic rate of reproduction 'r' of *B. angularis* for the best salinity x feed concentration with *C. ellipsoidea* (4x10⁶ cells mL⁻¹), *A. convolutus* (3 x 10⁶ cells mL⁻¹), *S. protuberans* (1 x 10⁶ cells mL⁻¹), and *C. infusorium* (4x10⁶ cells mL⁻¹) at the optimal temperature were 1.74, 1.57, 0.92 and 1.56. Based on the study, the maximum reproductive potential of *B. angularis* can be achieved by feeding with *C. ellipsoidea* at a salinity and temperature of 0.5% and 29 ± 1 °C, respectively, which is useful for larviculture of fishes.

Keywords: Reproductive rate, *Brachionus angularis*, *Chlorella ellipsoidea*, *Ankistrodesmus convolutes*, *Scenedesmus protuberans*, *Chlorococcum infusorium*.

1. Introduction

Rotifers serve as food resources to fish and crustacean larvae due to their small size, shape and slow mobility [1]. They are cultured in high densities and also produce resting eggs in adverse conditions [2]. In addition, these zooplankters are nutritionally rich with all essential nutrients and can be enriched to meet the nutritional requirement of the fish larvae [3]. A number of rotifers such as *Brachionus plicatilis*, *B. rotundiformis*, *B. calyciflorus*, *B. angularis*, *B. rubens* and *B. patulus* are used as live food for various marine, estuarine and freshwater larval fishes or even in rearing brackishwater crustaceans [3, 4].

An important factor that determines the effectiveness for mass culture of rotifers is its intrinsic rate of reproduction [5]. The reproductive rate 'r' of rotifer constitute the totality of all life table parameters, because it combines survival, fecundity and the timing of development and reproduction of rotifers [6]. In natural environmental conditions, presence and abundance of rotifers depends on both abiotic (e.g. temperature, salinity) and biotic factors (e.g., food availability, predation) and variations in environmental conditions [1]. The major factors that influence the reproductive rate of rotifers are temperature [7, 8], salinity of culture medium [9], food quality and quantity [10-13].

Salinity plays a vital role in the reproductive potential of an individual rotifer. Total dissolved salts and relative specific ionic concentrations are more important factors responsible for conditioning rotifers in the nature [14, 15]. The influence of salinity is directly related to the osmotic regulation of an individual, which in turn is strongly dependent upon the genotype and the species [10]. Temperature seems to be another main factor that determines the reproductive rate of rotifers thereby affecting species composition and richness of rotifers in natural water bodies [16].

The feed type, feed concentration and particle size plays a vital role in influencing the population rates and other life-history parameters of rotifers [11]. The particle size of the feed had been a significant influence on the ingestion by rotifers that in turn is directly related to the reproduction and growth of the individual. The particle size preference varies with the rotifer species [17, 18].

The population biological study on Indian rotifers has received very little attention till date even though they formed a dominant component in both the fresh and brackishwater plankton samples [19]. The determination of the reproductive potential for a specific rotifer species under controlled conditions is the key for developing mass culture of the potential rotifer species for rearing many finfish larvae [20]. Hence, the aim of this study was to evaluate the reproductive rate ' r ' of *B. angularis* against different temperature, salinity, feed and feed concentrations.

2. Materials and methods

2.1 Rotifer

Veli Lake (latitudes 8°25'-8°35'N & longitudes 76° 50' 76° 58'E), is a freshwater lake situated approximately 5Km North-West of Thiruvananthapuram, Kerala, India with a length of 1 km long. During the south west monsoon, the lake opens to the sea through a narrow outlet and seawater exchange takes place during this time. *Brachionus angularis* (Length 80-110 μm ; width 76-88 μm) collected from the aforesaid lake were employed for the study by using a starter culture of amictic female. The cultures were fed with *Chlorella ellipsoidea* and maintained at room temperature (29 \pm 1 °C) under continuous fluorescent illumination (1000 lux).

2.2 Design of experiment

Salinity, temperature and microalgal diets at different concentrations of the algae were the variables tested. Based on a series of preliminary salinity tolerance tests, three salinity levels (0.5%, 5.0% and 10.0%) were selected and the required salinities were prepared by diluting sterile saline water with distilled water. The three different temperatures selected were 25 \pm 1 °C, 29 \pm 1 °C and 35 \pm 1 °C, maintained by thermostatically controlled water baths under diffused and continuous fluorescent illumination (1000 lux).

Four algal diets, *Chlorella ellipsoidea*, *Ankistrodesmus convolutus*, *Chlorococcum infusorium*, and *Scenedesmus protuberans* were employed for the study. The stock cultures of *C. ellipsoidea* and *A. convolutus* were provided from the Vizhinjam Research Centre of CMFRI, Kerala (INDIA) while, *S. protuberans* and *C. infusorium* were isolated from Veli Lake by serial dilution method [21]. All the algal cultures were maintained in the laboratory by using Walne's medium under constant illumination. The required cell densities were prepared by centrifuging the algal cultures at 3000 g and then introduced the cells into the experimental vials [15 ml] of appropriate salinities. The experimental feed concentrations employed were 1 x 10⁶, 2 x 10⁶, 3 x 10⁶, 4 x 10⁶, 6 x 10⁶, 8 x 10⁶, 10 x 10⁶ and 12 x 10⁶ cells mL⁻¹ for all the algal diets. The algal cell counts were taken with a hemocytometer.

The rotifers were acclimatized to the experimental salinities, temperature and feed concentrations for one month prior to the experiment. Five ml of the medium of each feed type with appropriate feed concentrations and salinities corresponding to the rotifer species were taken in vials. Five numbers of previously acclimatized amictic females (with a single egg) of *B. angularis* (one animal per one ml) was carefully transferred to the appropriate medium with a micropipette. A total of 216 vials were set up for each feed as follows: 1 feed type x 8 feed concentrations x 3 salinities x 3 temperatures x 3 replicates. After three days (72 hours incubation), the experimental vials were fixed using 4% formaldehyde solution. The final populations of rotifers were estimated by counting the animals under a stereomicroscope with an overall magnification of 40X. Intrinsic rate of reproduction (r) was estimated using the

following formula [6].

$$r = \frac{\ln(Nt) - \ln(N_0)}{t}$$

Where, r = instantaneous growth rate; $\ln(Nt)$ = natural logarithm of population density after time t ; $\ln(N_0)$ = natural logarithm of initial population; t = duration of experiment (3 days).

2.3 Statistical analysis

Each set of experiment was carried out in triplicate and the mean values were taken. Two-way ANOVA was used to compare the reproductive potential in relation to salinity and feed concentration against three temperatures. A three-way ANOVA was applied to evaluate the independent as well as interactive effects of temperature, salinity and feed concentration on the reproductive potential of *B. angularis*. All analyses were done using SPSS 14.0.

3. Results

The intrinsic rate of reproduction in relation to salinity, temperature and feed concentration using four feed types viz., *C. ellipsoidea*, *A. convolutus*, *C. infusorium*, and *S. protuberans* are presented below.

3.1 *C. ellipsoidea*

The intrinsic rate of reproduction, $r \pm \text{SD}$ of *B. angularis* fed with *C. ellipsoidea* against three different salinity levels and temperature are depicted in Fig 1A-C. The optimum ' r ' value was found at room temperature (29 \pm 1 °C) and at salinity (0.5%) for all the feed concentrations. At low temperature (25 \pm 1 °C), r was greatly influenced by salinity (F=15.060; $p < 0.001$) and concentration of the algae (F= 15.751; $p < 0.001$) while in combination of both parameters there was no marked (F= 1.623; $p > 0.001$) effect on the reproduction (Fig 1A). Meanwhile, at room temperature (29 \pm 1 °C), salinity (F= 29.622; $p < 0.001$), and feed concentration (F= 32.768; $p < 0.001$) exert significant influence on the r both independently and in combination (F= 5.563; $p < 0.001$) (Fig 1B). During the exposure to higher temperature (35 \pm 1°C), salinity (F= 14.218; $p < 0.001$) and feed concentration (F= 23.325; $p < 0.001$) prominently determines the intrinsic rate of reproduction of the rotifer while in combination these factors have no effect (F= 2.008; $p > 0.001$) (Fig 1C).

Salinity plays an inverse relationship with reproduction of *B. angularis*. The density of rotifers at lower temperature reduced from 52 \pm 14 to 18 \pm 2 as the salinity increased from 0.5 ‰ to 10.0%. Meanwhile, the maximum numbers of individuals (194 \pm 62) were observed at a salinity of 0.5% and which was reduced (34 \pm 14) with further elevation in salinity to 10.0% at optimum temperature (29 \pm 1 °C).

The concentration of *C. ellipsoidea* fed to *B. angularis* also plays a major role in determining the r value. The intrinsic rate of reproduction increased with increase in the density of algae from 1x10⁶ to 4x10⁶ cells mL⁻¹ and decreased with further elevation in the levels of feed, irrespective of temperature and salinity. Therefore, the optimum level of *C. ellipsoidea* required for r_{max} were found to be 4x10⁶ cells mL⁻¹.

The results of three-way ANOVA, comparing the reproductive potential of *B. angularis* in relation to salinity, temperature and concentration of *C. ellipsoidea* showed that all factors greatly influence the reproductive rate of rotifer and is shown in Table 1.

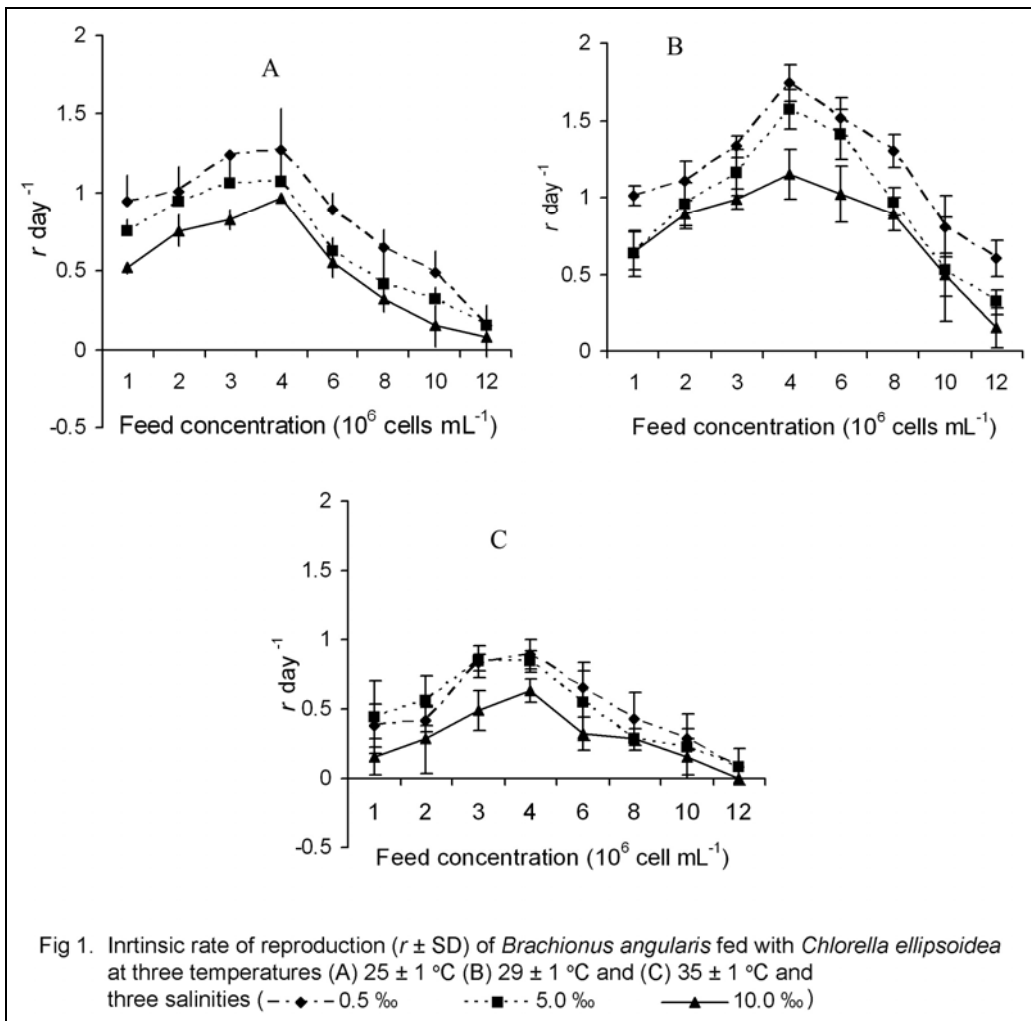


Fig 1. Intrinsic rate of reproduction ($r \pm SD$) of *Brachionus angularis* fed with *Chlorella ellipsoidea* at three temperatures (A) 25 ± 1 °C (B) 29 ± 1 °C and (C) 35 ± 1 °C and three salinities (---◆--- 0.5 ‰ ---■--- 5.0 ‰ —▲— 10.0 ‰)

Table 1: Three-way ANOVA, comparing the reproductive potential of *B. angularis* in relation to salinity (S), Feed Concentration (FC) and Temperature (T) against *Chlorella ellipsoidea*.

Sources of variation/parameters	SS	Df	MS	F	P value
Salinity (S)	14419.60	2	7560.15	46.80	0.000**
Feed concentration (FC)	52921.07	7	7209.80	49.07	0.000**
Temperature (T)	37707.62	2	18853.81	122.38	0.000**
S x FC	15167.22	14	1083.37	7.03	0.000**
FC x T	44002.09	14	3143.00	20.40	0.000**
S x T	10662.04	4	2665.51	17.30	0.000**
S x FC x T	16518.25	28	589.94	3.82	0.000**
Error	22184.66	144	154.06		

3.2 A. convolutus

The reproductive potential, $r \pm SD$ of *B. angularis* fed with various densities of *A. convolutus* at three salinity levels is shown in Fig 2 A-C. The optimum salinity and temperature required for r_{max} of *B. angularis* fed with *A. convolutus* were found to be 0.5% and 29.0 ± 1 °C. The ‘ r ’ value of the rotifer exposed to low temperature (25 ± 1 °C) showed significant influence to salinity ($F=20.610$; $p<0.001$), feed concentration ($F=57.630$; $p<0.001$) and in combination ($F= 4.952$; $p<0.001$) of salinity and feed level (Fig 2A). Similarly, the reproductive rate was prominently decreased in *B. angularis* exposed to various levels of salinity ($F=22.639$; $p<0.001$), density of algae ($F=34.010$; $p<0.001$) and in combination of both ($F=3.057$; $p<0.005$) at optimum temperature (Fig 2B). Meanwhile, at high temperature (36 ± 1 °C), the ‘ r ’ value changed significantly with salinity ($F=16.020$; $p<0.001$) and concentration of *A.*

convolutus ($F=17.135$; $p<0.001$), independently while there was no marked ($F=0.951$; $p>0.001$) variation in the reproduction of rotifer in combination of these variables (Fig 2C).

Salinity influences the reproduction of *B. angularis* greatly. The optimum salinity required for r_{max} was found to be 0.5% and further increase in this variable resulted in reduced number of animals. The density of rotifer reduced from 113 ± 36 to 25 ± 14 , as salinity increased 20 times than the optimal level. The experiment showed that density of *A. convolutus* also determines the reproductive success of *B. angularis*. The ‘ r ’ value elevated from 0.73 ± 0.04 to 1.27 ± 0.09 in accordance with increase in concentration of diet from 1×10^6 to 3×10^6 cells mL^{-1} and further addition of algae as feed resulted in reduced reproductive rate, irrespective of two abiotic parameters.

Three way ANOVA comparing the reproductive potential of *B. angularis* in relation to salinity, temperature and feed concentrations showed that the intrinsic rate of reproduction

was prominently influenced by the above three factors and is shown in Table 2.

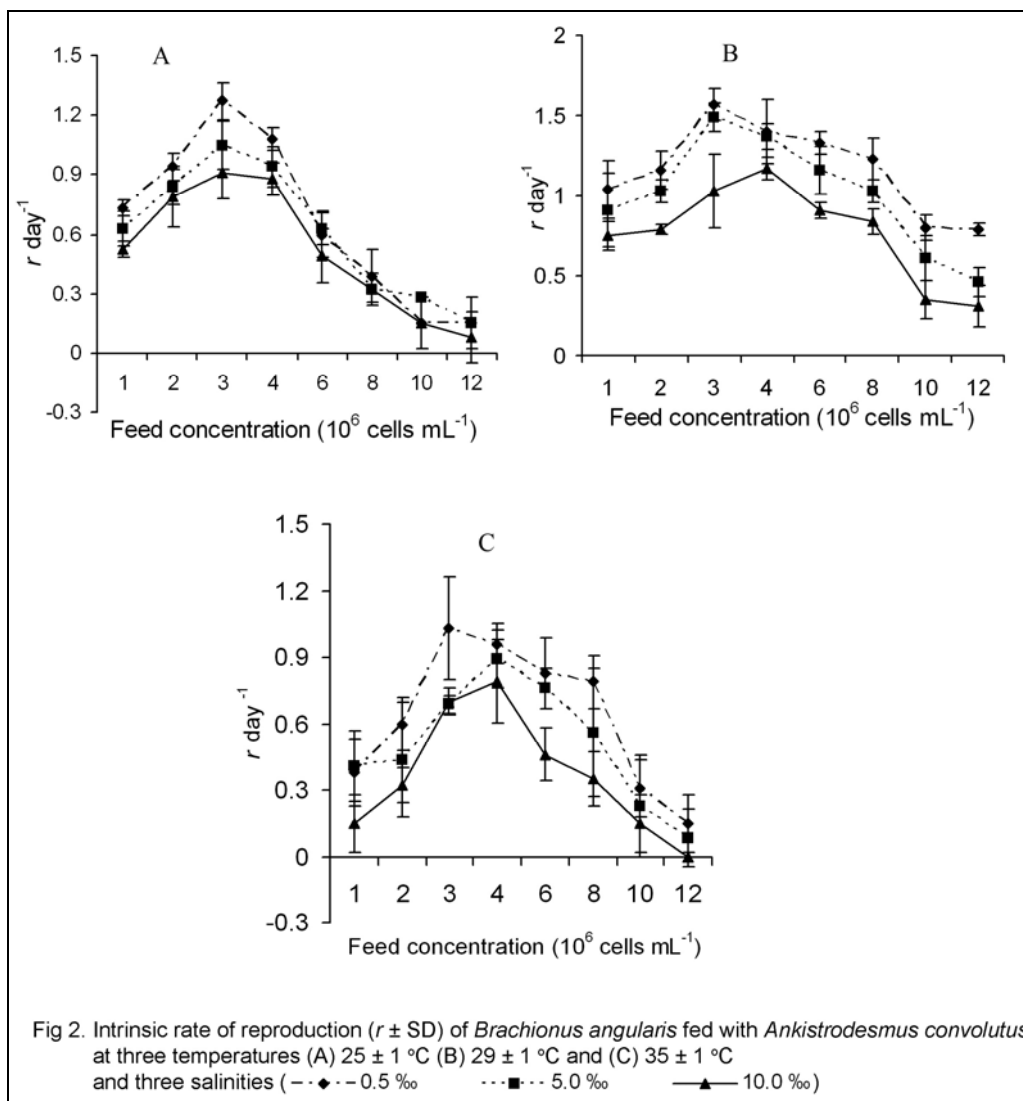


Table 2: Three-way ANOVA, comparing the reproductive potential of *B. angularis* in relation to salinity (S), Feed Concentration (FC) and Temperature (T) against *Ankistrodesmus convolutus*

Sources of variation/parameters	SS	df	MS	F	P value
Salinity (S)	6307.52	2	3153.76	43.55	0.000**
Feed concentration (FC)	33194.65	7	742.09	65.48	0.000**
Temperature (T)	16610.10	2	305.04	144.69	0.000**
S x FC	5247.00	14	364.78	5.18	0.000**
FC x T	19348.66	14	382.04	19.08	0.000**
S x T	3268.47	4	817.11	11.28	0.000**
S x FC x T	4026.12	28	143.79	1.98	0.005*
Error	10427.73	144	72.41		

3.3 *S. protuberans*

The specific growth rate ' $r \pm SD$ ' of *B. angularis* in different regimes of salinity, temperature and feed concentrations of *S. protuberans* are shown in Fig 3A-C. The highest ' r ' value for this rotifer fed with *S. protuberans* was obtained at a temperature of 29 ± 1 °C and a salinity of 0.5%. The feed concentration of algae showed significant variation in intrinsic rate of reproduction under the influence of low salinity ($F= 24.208$; $p< 0.001$) (Fig 3A) and optimum ($F= 23.110$; $p< 0.001$) (Fig 3B) temperatures. Meanwhile, at higher

temperature (35 ± 1 °C), the reproductive rate ' r ' was significantly influenced by salinity ($F= 21.966$; $p< 0.001$), feed concentration ($F= 134.138$; $p< 0.001$) and in combination ($F= 11.325$; $p< 0.001$) of both these variables (Fig 3C). An interesting observation found during this experiment was the influence of algal concentration (1×10^6 cells mL⁻¹) required to obtain the maximum ' r ' value, irrespective other two factors and further increase in the level of algae resulted in reduced intrinsic rate of reproduction.

Three way ANOVA comparing the reproductive potential of

B. angularis in relation to salinity, feed concentration and temperature showed that three parameters namely salinity, temperature and algal concentration significantly ($p < 0.001$) influence the reproductive rate of *B. angularis*, while salinity

X temperature ($F = 1.145$; $p > 0.001$) and salinity X temperature X feed concentration ($F = 0.722$; $p > 0.001$) has no effect on reproductive rate of *B. angularis*.

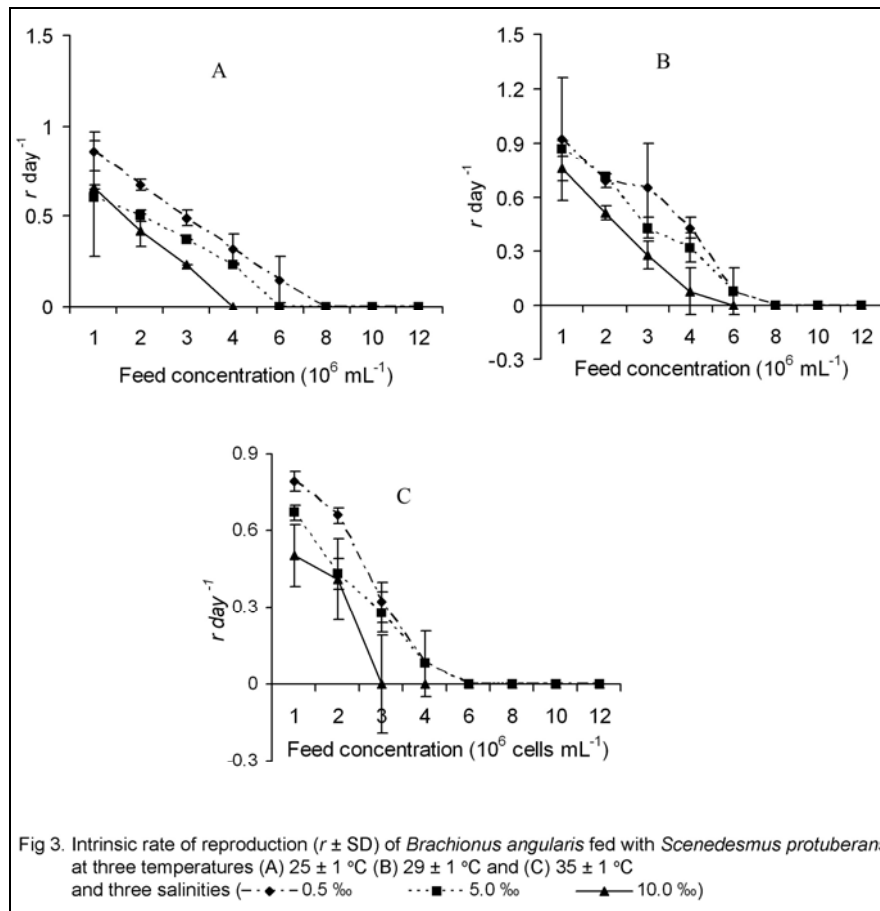


Table 3: Three-way ANOVA, comparing the reproductive potential of *B. angularis* in relation to salinity (S), Feed Concentration (FC) and Temperature (T) against *Scenedesmus protuberans*

Sources of variation/parameters	SS	df	MS	F	P value
Salinity	118.39	2	297.68	14.58	0.000**
Food concentration	2083.75	7	59.19	3.31	0.000**
Temperature	100.34	2	50.17	12.35	0.000**
Salinity x FC	179.82	14	12.84	3.16	0.000**
Food con x Temp	220.76	14	15.76	3.38	0.000**
Salinity x Temp	18.06	4	4.65	1.14	0.338
Salinity x FC x Temperature	82.06	28	2.93	0.72	0.843
Error	584.66	144	4.06		

3.4 *C. infusorium*

The intrinsic rate of reproduction $r \pm SD$ of *B. angularis* at different salinities and feed concentrations of *C. infusorium* exposed to different temperatures are shown in Fig 4A-C. The reproductive behavior of *B. angularis* depends greatly on temperature of the ambient medium. At lower temperature, the 'r' value of rotifer was significantly affected by salinity ($F = 35.393$; $p < 0.001$), concentration of the algae ($F = 35.518$; $p < 0.001$) and in combination ($F = 5.531$; $p < 0.001$) of these variables (Fig 4A). Similar results were observed at room temperature (29 ± 1 °C) where salinity ($F = 53.139$; $p < 0.001$), density of feed applied ($F = 46.625$; $p < 0.001$) and salinity x feed concentration ($F = 14.000$; $p < 0.001$) determines the reproduction of rotifers (Fig 4B). Meanwhile, in higher temperature (36 ± 1 °C), the intrinsic rate of reproduction was

prominently affected by salinity ($F = 6.634$; $p < 0.005$), density of algal feed ($F = 17.520$; $p < 0.001$) and both salinity x feed concentration ($F = 5.114$; $p < 0.001$) (Fig 4C).

Irrespective of temperature and feed concentration, the reproductive rate of *B. angularis* decreased with increase in salinity. This is evident from the number of rotifers produced during optimum conditions of salinity and temperature, where the density reduced from 116 ± 27 to 19 ± 4 individuals at elevated levels of salinity from 0.5% to 10.0%. The concentration of *C. infusorium* fed to *B. angularis* also determines the r . The r_{max} were observed at a density of 4×10^6 cells mL^{-1} and further increase in concentration of feed resulted in reduced reproductive rate of rotifer. Interestingly, the specific growth rate of *B. angularis* was found to be higher at an elevated (35 ± 1 °C) temperature than lower temperature,

while it was found to be higher at lower temperature in all other experiments. Three-way ANOVA comparing the reproductive potential of *B. angularis* in relation to salinity, temperature and feed

concentrations of *C. infusorium* showed that the intrinsic rate of reproduction was prominently influenced by the above three factors and is shown in Table 4.

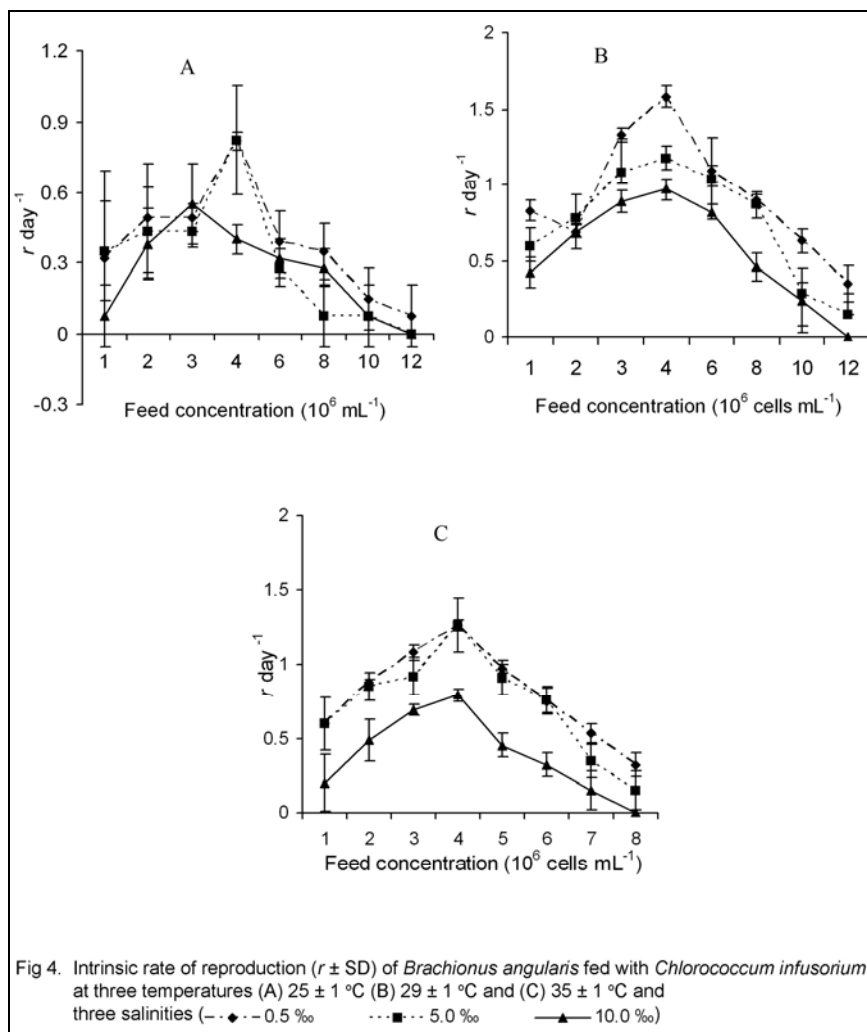


Fig 4. Intrinsic rate of reproduction ($r \pm SD$) of *Brachionus angularis* fed with *Chlorococcum infusorium* at three temperatures (A) 25 ± 1 °C (B) 29 ± 1 °C and (C) 35 ± 1 °C and three salinities (---◆--- 0.5‰ ---■--- 5.0‰ —▲— 10.0‰)

Table 4: Three-way ANOVA, comparing the reproductive potential of *B. angularis* in relation to salinity (S), Feed Concentration (FC) and Temperature (T) against *Chlorococcum infusorium*.

Sources of variation/parameters	SS	df	MS	F	P value
Salinity (S)	5594.56	2	2797.28	87.86	0.000**
Feed concentration (FC)	19903.21	7	2843.31	89.30	0.000**
Temperature (T)	7819.89	2	3909.94	122.88	0.000**
S x FC	8033.43	14	573.81	18.02	0.000**
FC x T	8672.54	14	619.46	19.45	0.000**
S x T	3343.18	4	835.79	26.25	0.000**
S x FC x T	7146.59	28	255.23	8.01	0.000**
Error	4584.66	144	31.83		

4. Discussion

The population growth rate or intrinsic reproductive rate (r) of rotifers indicates information regarding the changes in the reproductive performances under stress conditions and provides the most suitable environmental conditions for reproduction [11, 22]. Rotifers in general show high intrinsic rate of reproduction than other zooplanktors due to its small size and parthenogenic mode of reproduction [6]. Therefore, various biotic and abiotic factors like temperature, salinity, feed type and feed concentration exert prominent influence on this parameter [23]. The data on the rate of population increase (r

day⁻¹) provides information about population dynamics in several life table and demographic studies [10, 12, 24]. In addition, this variable also helps to evaluate the abiotic and biotic requirements for rotifer mass culture systems [25]. In our study, r value of *B. angularis* was strongly affected by salinity, temperature, feed type and feed concentration. Salinity is an important abiotic variable strongly determines the abundance and diversity of rotifers [14, 15]. Studies concerning the impact of salinity on r are mainly concentrated on few euryhaline species such as *B. plicatilis* and *B. rotundiformis* while little information is available on *B.*

angularis. In our results, r value of *B. angularis* showed an inverse relationship with salinity with the maximum at 0.5‰ and reduced with further increase in salinity level till 10‰ [26]. *B. angularis* was reported to be the most salt tolerate freshwater rotifer with a salinity tolerance level of 0.5-24‰ [27]. The possible physiological mechanisms involved in the lower salinity tolerance of freshwater rotifers can be due to the failure of osmoregulation, reduced swimming rate, less hatching success, mass mortality of neonates and biochemical changes [10]. The influence of salinity is directly correlated with the osmoregulatory capacity of the rotifers, which in turn is strongly dependent on the species genotype that is adapted to an optimum salinity in which r is high [14,26].

Temperature has a direct effect on reproductive rate of *B. angularis* and the highest r value was registered at room temperature (29 ± 1 °C). In an earlier report, the maximum specific growth rate (0.80 ± 0.054) of *B. angularis* was observed at a temperature of 35 °C [26]. A possible reason for this variation with our results (29 ± 1 °C) could be the strain difference used in the study. The survival or reproductive performance differs between the strains of the same species in response to temperature as reported in Anging strain of *B. calyciflorus* [28]. However, the direct effect of temperature on r of rotifers is difficult to determine since it is mainly affected by population density and food supply [26]. Furthermore, the influence of temperature on the intrinsic rate of reproduction could also be attributed to the variation in developmental stages or metabolic activities of rotifer [14].

In order to optimize the culture conditions for obtaining a maximum production of *B. angularis*, effects of feed and feed selectivity should be determined. Among the various groups of phytoplankton, green algal species are widely used for the culture of rotifers [29] and the reproductive rate depends on the type of algae employed for its production [30]. In the present experiment, *B. angularis* attained the highest intrinsic rate of reproduction with *C. ellipsoidea* followed by *A. convolutus*, *C. infusorium* and *S. protuberans*. The most commonly used microalgae as food for Brachionid rotifers are *C. vulgaris* and *N. oculata* [31] and *Chlorella* is preferred to be best among unialgal diets [32]. For *B. angularis*, *C. ellipsoidea* ($r=1.74$) were found to be a superior feed based on the specific growth rate than *Chlorella vulgaris* ($r=0.801$) [26]. The main factors that influence the suitability of microalgae as food for rotifer include size, shape, digestibility, motility and nutrient composition [17]. The preference to *C. ellipsoidea* by *B. angularis* in the study could be attributed to its small size (2 - 5 µm) compared to *C. infusorium* (2 - 7 µm), *A. convolutus* (10 - 20 µm) and *S. protuberans* (30 - 37µm). This proposal is further proved by the least ' r ' values obtained on the diet of *S. protuberans*, due to the particle size of this alga being too big for the ingestion by this rotifer.

B. angularis attained the maximum r at different concentration of *C. ellipsoidea* (4×10^6 cell mL⁻¹), *A. convolutus* (3×10^6 cells mL⁻¹), *C. infusorium* (4×10^6 cells/ml) and *S. protuberans* (1×10^6 cells mL⁻¹). The concentration of the feeds had a significant influence on r , which is dependent on the size of the algal diets [34] and the ingestion capacity of the rotifers [33]. Earlier reports on various rotifers suggests that ingestion rates increase with increasing concentration of microalgae, reach at high densities at a certain level [30] and then remain stable or decrease [31,34]. *B. angularis* showed a similar kind of pattern against four different algae used in our study. This feeding behavior by rotifers at high concentration of microalgae could be due to the toxic effect of algae on rotifers [35], interfere with the feeding apparatus of rotifer [36],

inhibit the ciliary activity, thereby leading to reduced swimming speeds [37] or reduced assimilation rate due to rapid movement of food through gut, eventually leading to malnutrition [38]. The mechanism for accepting food particles involves the cilia of the pseudotrochus, the buccal funnel, the mastax jaw, and the feeding behaviors as well [39]. Earlier reports suggested that that *B. angularis* prefer food items ≤ 5 µm in mean diameter; whereas *B. calyciflorus*, *B. rubens* and *B. quadridentatus* were preferred food items ≤ 15 µm in mean diameter [18].

In conclusion, *B. angularis* attained optimum maximum r at salinity (0.5‰) and temperature (29 ± 1 °C) with *C. ellipsoidea* as feed at a level of 4×10^6 cells mL⁻¹. Determination of optimum abiotic and biotic variables for maximum r is required for mass production and the application of *B. angularis* for larviculture of freshwater ornamental fishes with small larvae. Moreover, *B. angularis* can also be used as a suitable candidate live feed for fish larvae due of its smaller size (80 -120 µm) and tolerance to salinity.

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