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The Impacts of Different Algal Diets, Temperature, and Salinity on Locally Isolated Euryhaline Rotifer's (*Brachionus plicatilis*) Growth and Egg Production

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ABSTRACT

The aquaculture industry commonly uses the euryhaline rotifer *Brachionus plicatilis* as food for various marine fish larvae. The current study examined the growth and/or egg production rates of *B. plicatilis*, isolated from the Nile River's Damietta estuary, considering temperature, salinity, and the concentrations of three algal diets. The microalgae species, *Tetraselmis* sp., *Nannochloropsis* sp., and *Chaetoceros* sp., were used as feed with various densities ranging from 2×10^4 to 6×10^5 cells/mL. The population density, specific growth rates (SGRs), and egg production rate (EPR) were significantly affected ($P<0.01$) when fed diets of different densities. The highest population growth of 503±55.15 individuals/mL, SGR of 0.34±0.10, and EPR of 2.31±0.13 were recorded for the *Tetraselmis* sp. at a concentration of 6×10^5 cells/mL. The temperature showed a significant ($P<0.05$) effect on growth rate and EPR. The rotifers cultured at 20°C had a higher growth rate than those grown at other temperatures. EPR was also expected to increase with increasing temperatures, except for 30°C. *B. plicatilis* exhibited a greater rate of population growth at salinities ranging from 15 to 20psu compared to a salinity of 30psu. It was deduced that variations in temperature and salinity affect the development rate and reproductive activity of *B. plicatilis*. The best food for *B. plicatilis* was *Tetraselmis* sp., followed by *Nannochloropsis* sp., while the *Chaetoceros* sp. seemed to be insufficient.

INTRODUCTION

Marine hatcheries commonly use rotifers as the initial live food for a wide variety of fish species due to their small size **(Tanaka *et al.*, 2005)**, high survival rate **(Sahandi & Jafaryan, 2011)**, and ability to culture at a high density with a low cost **(Hagiwara *et al.*, 2001)**. In the Damietta estuary of the Nile River, large populations of the Brachionidae rotifers are usually present in spring and early summer, where peak values of more than 100 individuals per liter may occur (**El-Tohamy *et al.*, 2018**).

Euryhaline rotifers, specifically the *Brachionus* spp., are currently indispensable as live food in the aquaculture industry **(Hagiwara *et al.*, 1996; Fielder *et al.*, 2000)**. The genus *Brachionus* has a total length ranging from 100 to 400µm. It is available in three major types: *B. plicatilis*, Type-L (the large type), with 190–320µm in length, *B.*

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rotundiformis Type-S (small type), with 140–220µm, and *B. rotundiformis* (super small type) Type-SS, with 100–160µm **(Segers, 1995)**. Fish with different mouth sizes require the availability of small and large rotifers **(Hagiwara *et al.*, 2007)**; remarkably, providing the ideal prey size for selection by various larval stages and/or species would enhance hatchery production **(Kuronuma & Fukusho, 1984)**. In order to secure marine fish fries and lessen the impact on natural resources, Egypt has recently developed numerous marine hatcheries **(Abdel-Hady *et al.*, 2024)**. As a primary food source for countless species of marine fish larvae in these hatcheries, especially the gilthead seabream (*Sparus aurata*) **(Eid *et al.*, 2018)** and the European sea bass (*Dicentrarchus labrax*) **(El-Dakar *et al.*, 2001)**, the L-type and S-type rotifers are currently leading the market. In the current study, the rotifers collected were of the large L-type of *B. plicatilis* (Fig. 3).

Like other Monogononta, the rotifer *B. plicatilis* can reproduce asexually or sexually depending on a variety of conditions, including food type, water temperature, salinity, and the overall quality of the water **(Lubzens *et al.*, 1989; Gilbert, 2020)**. It reproduces asexually under suitable conditions by producing one or two eggs of 80–130µm every few hours **(Abd Rahman *et al.*, 2018)**. In a mass culture system, it is crucial to prevent variables that promote sexual reproduction in order to produce rotifers in large quantities and assure the prevalence of asexual reproduction **(Theilacker & McMaster, 1971; Dhert *et al.*, 2001)**. **Sarma *et al.* (2005)** found that temperature impacts both the rate of egg production and the hatching times of parthenogenetic eggs, which in turn affects the egg ratio. Consequently, in order to determine the optimal temperature, a part of this study was designed to investigate the effect of temperature variations on the *B. plicatilis* egg production.

Microalgae serve as the primary food source for most cultured rotifers. Microalgae production is essential for healthy, nutritious rotifer production **(Lubzens *et al.*, 2001; Abd Rahman *et al.*, 2018)**. According to **Wikfors and Ohno (2001)**, the microalgae species most frequently used in the rotifer culture include *Tetraselmis*, *Nannochloropsis*, *Chaetoceros*, and *Isochrysis*. *Nannochloropsis* is the most commonly used live food for most of the rotifer species due to its ability to be cultured in high densities due to its high lipid content **(Alam & Shah, 2004; AbdRahman *et al.*, 2018)**. The objective of this study was to assess the impact of various concentrations of the microalgae *Tetraselmis* sp., *Chaetoceros* sp., and *Nannochloropsis* sp. on the growth and reproductive performance of locally isolated euryhaline *B. plicatilis*. The effect would be evaluated by measuring a quantitative indicator, such as the daily growth and egg production rates, and examining how the utilization of varied food densities influences these rates.

Several factors influence the size, optimal growth rate, nutritional value, and the reproduction of the rotifers. In addition, the differences in temperature, salinity, and

strain impact the rotifer's productivity significantly **(Lubzens & Minkoff, 1988)**. For example, the Japanese (S-type) occurs in the summer and is most productive at high temperatures, while the large-size strain (L-type) predominates in the winter to be most productive at lower temperatures **(Lubzens *et al.*, 1989)**. It was also indicated in many earlier studies that temperature levels directly influence the somatic cell growth and egg production of numerous rotifer species **(Sarma, 1989; Stelzer, 2002)**. Salinity is crucial in determining the adaptation of the rotifers in their natural habitat **(Anitha *et al.*, 2015)**. Additionally, salinity variations can significantly influence the rotifer biological process, leading to growth, reproduction, and population density changes **(Lee *et al.*, 2022)**. According to the study conducted by **Pan *et al.* (2016)**, both lower salinity levels of 5psu and higher salinity levels ranging from 30 to 35psu can hinder the reproductive activity of certain invertebrates.

Despite being a prominent part of the brackish water zooplankton samples, the population biology study on the Egyptian rotifers has received little attention. It is crucial to ascertain the population growth rate of a locally isolated rotifer species in controlled conditions to establish a mass culture of this rotifer species for rearing fish larvae. The primary objective of this study was to assess the rates of development and egg production in *B. plicatilis* at varying temperatures and salinity levels while being fed different algal diets of different densities.

MATERIALS AND METHODS

1- Algal culture

Pure cultures of local strains of the three algal species (*Nannochloropsis* sp., *Tetraselmis* sp., and *Chaetoceros* sp.) were obtained from the National Institute of Oceanography and Fisheries of Alexandria, Egypt and were cultured in a batch-culture system. The microalgae ranges were from 2– 3µm for *Nannochloropsis* sp.,~10– 12µm for *Tetraselmis* sp., and 4– 6µm for *Chaetoceros*sp. The algae were cultured at 21 ±1°C, 25psu salinity, 16 h:8 h of light (2500 lux), and the dark cycle in water was enriched with F/2 medium. The algae were harvested during the exponential phase, centrifuged at 2000rpm for 8min, and concentrated to $1–2 \times 10^8$ cells/ml at 4°C in a refrigerator following the outlined data of **Coutteau (1996)**.

2- The rotifer stock culture

Samples of mixed zooplankton were collected from the water of the Damietta estuary using a plankton net with a mesh size of 65µm and a mouth diameter of 0.5m. In the lab, samples were screened through a 500µm mesh net to eliminate fish and decapod the larvae, and subsequently were filtered through a 120-µm mesh to remove the protozoa and nauplii of the copepods and barnacles. Amictic females of *B. plicatilis* with loricae length of 110– 230µm were isolated from the remaining zooplankton to establish a stock culture through a batch culture technique, starting from a one-liter conical flask to 20-liter carboys. Seawater was filtered using a <10 µm pore size filter

paper. Temperature ranged from 15– 20°C, salinity from 20– 25psu, the pH fluctuated from 7.5– 8.5, dissolved oxygen from 5– 6.5mg/ l, and photoperiod: 12h light/12h dark during rearing. The experimental rotifers were fed a mix of *Tetraselmis* sp., *Nannochloropsis* sp., and *Chaetoceros* sp.

3- Assessment the suitability of different algal species for *B. plicatilis*

At a temperature of 20±2°C, culture water with a salinity of 20±2psu was prepared, and rotifers were inoculated in one liter Erlenmeyer flask filled with 500 ml of filtered seawater at a density of 2 individuals/ mL. Microalgae species were given to each rotifer culture at concentrations of 2×10^4, 1×10^5, 2×10^5, 3×10^5, 4×10^5, 5×10^5, and 6×10^5 cells/ mL. The test containers were subjected to fluorescent light with less than 200 lux intensity to ensure the microalgae survival while inhibiting their reproduction **(Abd Rahman *et al*., 2018)**. Aeration is necessary to prevent algae aggregation and enhance its accessibility to the rotifers. All conditions, except the algal densities, were maintained at constant levels. Using a hemocytometer, the number of the algal cells was counted every day. Then, the right amount of the algal cells was supplied to the culture containers to maintain the target cell concentrations. Every two days, 50% of each container's volume was replaced with fresh culture media injected with corresponding algal densities. Each day, four replicates of a 2ml sample from a homogenized rotifer culture were transferred using a pipette onto a Bogorov counting tray. Next, densities were measured by counting the rotifers and their eggs under a light microscope. Every treatment in this experiment was carried out in triplicate.

4- Culture conditions according to temperature and salinity

4.1. Temperature

The temperature experiments were conducted in an incubator manufactured locally with a cooling and heating system and internal lighting. The incubator was set at treatments of 10, 15, 20, and 25°C, separately. The rotifers were inoculated at 100 individuals/ mL density in a 100ml Erlenmeyer flask. The salinity of the culture water was kept at around 20±2psu, and 10% of the water was exchanged daily. The *Tetrasemis* sp. with a density of 6×10^5 cells/ml was given to the rotifers; the algae were supplied daily and counted using a hemocytometer **(Dhert, 1996)**. The rotifers were cultured for 16 days. Four replicates of a 1ml sample from a homogenized rotifer culture were pipetted daily onto a Bogorov counting tray. The rotifers and their eggs were counted under a light microscope to determine densities. In this experiment, all treatments were performed in triplicate. With the exception of the water's temperature, all parameters were kept as steady as possible.

4.2. Salinity

Culture waters with a 10, 15, 20, and 30psu salinity were prepared at 20±2°C. In a 100ml Erlenmeyer flask, the rotifers were inoculated at 100 individuals/mL density. Rotifers were fed with *Tetrasemis* sp. of a 6×10^5 cell/ml density; the algae were

counted using a hemocytometer and supplied daily. The rotifers were screened with 65-micron mesh every three days and then transferred to a new 100ml Erlenmeyer flask containing fresh culture water adjusted to the desired salinity. All conditions, except salinity, were maintained at constant conditions. All treatments were continued for 16 days in this experiment and conducted in triplicate.

5- Daily Growth Rate

The specific growth rate (SGR) was computed according to the equation described by **Rico-Martinez and Dodson (1992)** as follows:

$$SGR = (1/T)\ \text{In}(N_T/N_0)$$

Where, T is the number of culture days from the rotifers inoculation to the peak density; N_T is the final density after time T, and N_0 is the number of the rotifers in inoculation.

6- Number of eggs produced

According to the method described by **Rasdi *et al.* (2020)**, five egg-bearing females were isolated from the stock culture to a cultivation plate. Two hatched neonates were transferred to a new cultivation plate with the three algal diets at the seven concentrations mentioned previously. The quantity of eggs produced was measured every 12 hours, and each egg produced was removed after hatching the smaller size neonates. This experiment continued until all females died. In this study, the feeding experiment was done in three replicates.

Five neonates were transferred to 50ml transparent containers with 20ml of seawater at 20±2psu. The rotifers were fed with *Tetrasemis* sp. of a 6×10^5 cell/ml density. The containers were separately transferred to the incubator set at 5, 10, 15, 20, 25, and 30°C. The quantity of eggs produced was measured every 8 hours for only four days. All treatments in this experiment were carried out in triplicate.

The egg production rate (EPR) for the rotifer in this experiment was calculated using the formula given by **Edmondson (1965)** as follows:

$$EPR = E/D$$

Where, E is the number of eggs/females with eggs, and D is the day of the experiment.

7- Measurement of the area of neutral lipids

To examine the effects of temperatures and food type on neutral lipid accumulation *in vivo*, the Nile red (Sigma-Aldrich) staining was performed. The primary lipid fraction in the rotifer was neutral lipid, which contained most of the necessary fatty acids, including polyunsaturated fatty acids **(Birkou*et al.*, 2012)**. The rotifers were stained with the Nile red fluoresce to determine neutral lipid, and the total area of the stained lipid droplets was viewed under an epifluorescence microscopy **(Carman *et al.*, 1991)**. We made a stock solution of 12.5mg Nile red in 500ml acetone. The rotifer individuals were transferred to a Petri dish with 0.1ml of the Nile-red stock

solution and 4 ml of seawater. The rotifers were stained in the dark for 60 minutes and examined under an epifluorescent microscope (ZEISS, AX10). Neutral lipid droplets stained with the Nile red fluoresce became bright yellow **(Cole *et al*., 1990)**.

8- Statistical analysis

The data were expressed as mean values ±SD. One-way analysis of variance (ANOVA) with the Tukey test was used to compare the rotifer's productivity in relation to algal types, algal concentrations, salinity, and temperatures. Two-way ANOVA with Tukey was used to test the relationship between EPR with various algal types and different concentrations. All the data were subjected to a homogeneity test to satisfy the ANOVA at the level of significant differences ($P < 0.05$). All analyses were performed using SPSS 25 (SPSS Inc., Chicago, IL, USA).

RESULTS

1- The algal supply

The results were pronouncedly different among the algal species and between algal densities (Fig. 1). Promising results were obtained for *Tetraselmis* sp.compared to *Nannochloropsis* sp. and *Chaetoceros* sp. The growth of the rotifers *B. plicatilis* depending on *Tetraselmis* supply was the highest at 503±55.15 individuals/ mL on day 14 when 6×10^5 cells/mL was supplied; other experimental groups recorded lower growth rates. In the case of *Nannochloropsis* sp., the highest growth was at 105 individuals/mL on day 16 when 6×10^5 cells/mL was supplied, whereas, for *Chaetoceros* sp., the maximum growth rate was only at 66 individuals/mL on day 12 when 3×10^5 cells/mL was provided. The SGR significantly differed between different algal diets, and the highest was at 0.34±0.10 when 6×10^5 cells/mL of *Tetraselmis* was supplied (Fig. 2).

At the end of this experiment, the rotifers were stained with the Nile red to determine the amount of the stored neutral lipids when fed different algal diets. The results revealed that the *B. plicatilis* fed *Tetraselmis* sp. stored more lipids than those fed on *Nannochloropsis* sp. or *Chaetoceros* sp. (Fig. 3).

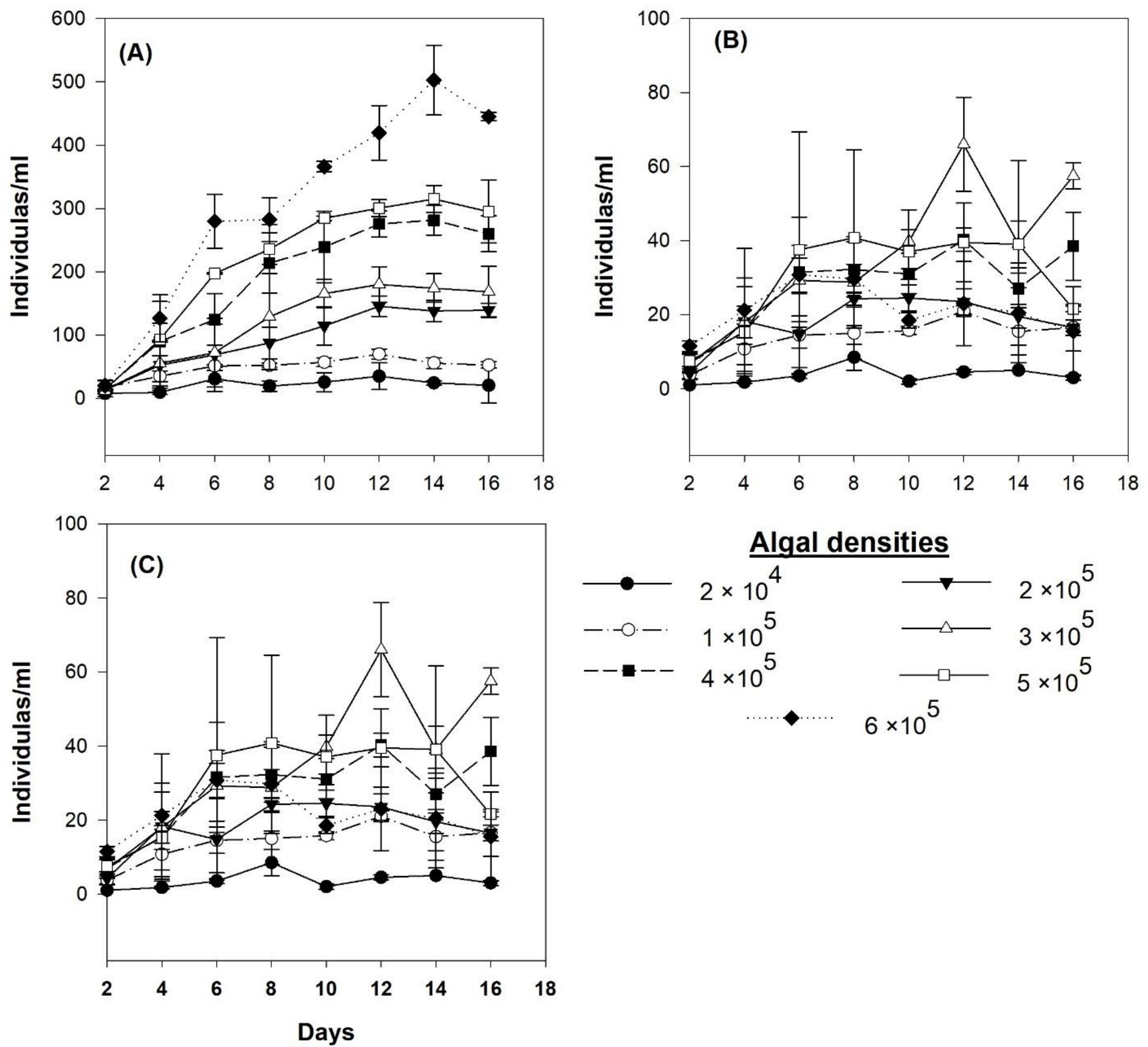

Fig. 1. Growth density of *B. plicatilis* at different densities of the three algal species (A=*Tetraselmis* sp., B= *Nannochloropsis* sp., and C= *Chaetoceros* sp.)

Values are represented as mean ± standard deviation (n = 3).

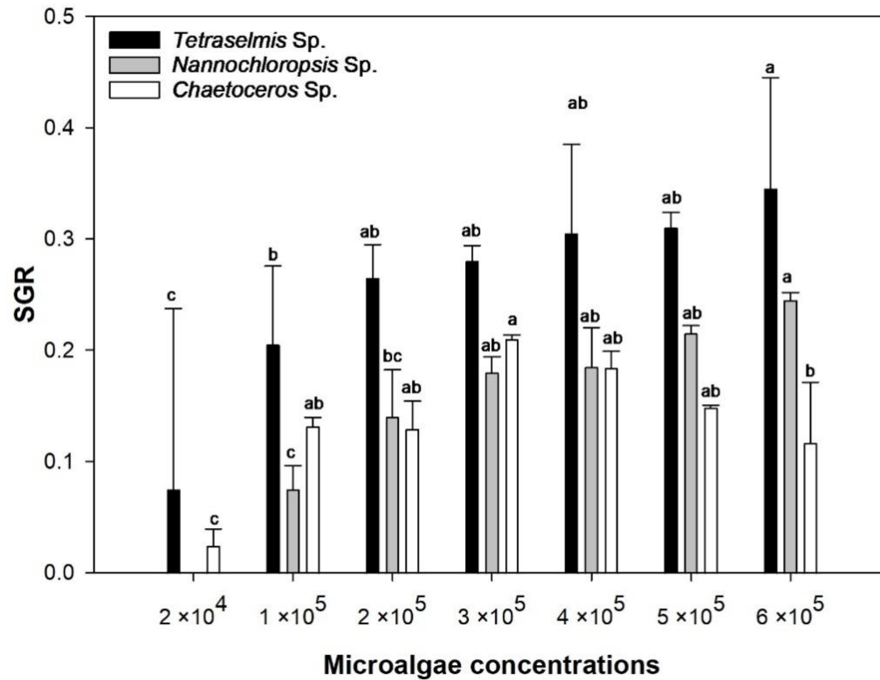

Fig. 2. Specific growth rate (SGR) of *B. plicatilis* at different densities of the three algal species Values are represented as mean ± standard deviation (n = 3). Different letters denote significant differences (P< 0.05).

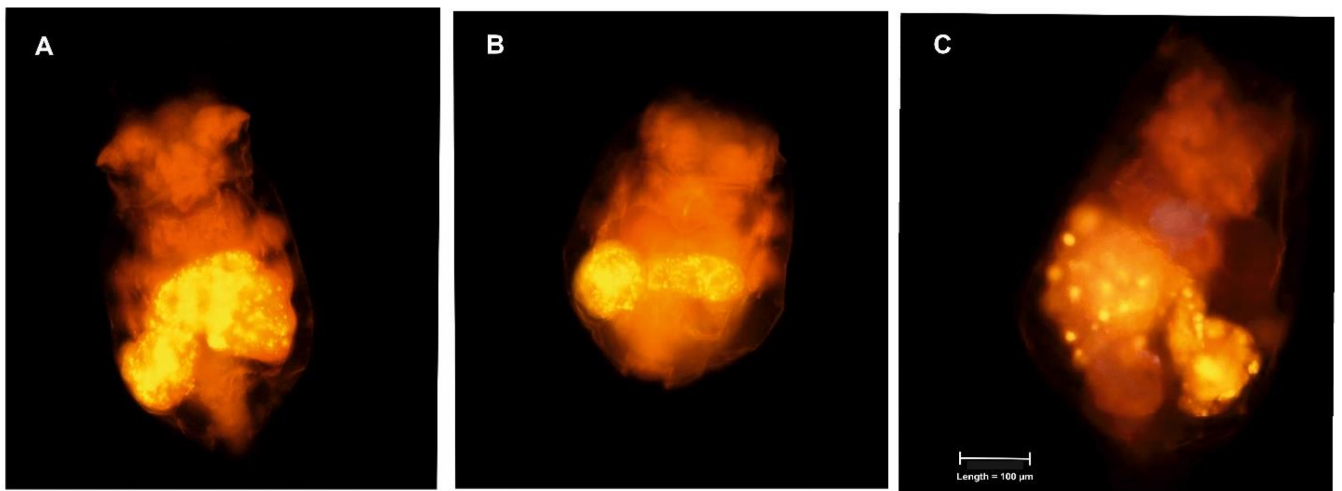

Fig. 3. *B. plicatilis* fed different diets (A= *Tetraselmis* sp., B = *Nannochloropsis*sp. and C = *Chaetoceros*sp.) stained with the Nile red

2. Effects of temperature and salinity on the rotifer growth

2.1.Temperature

Fig. (4) illustrates *B. plicatilis* growth at 10, 15, 20, 25°C. The rotifers cultured at 25 and 20°C reached the highest densities of 480±24.27 and 456±4.95 individuals/mL on

days 11 and 12; respectively, followed by a growth decline. The maximum density of 426±12.73 individuals/mL was achieved on day 13 at a temperature of 15°C, after which there was a decline in growth. Conversely, the population of the rotifers cultured at a temperature of 10°C exhibited a consistent and gradual increase in growth until day 15, reaching its peak density of 243±33.31 individuals/mL on that day. Compared to cultures grown at 20 and 25°C, *B. plicatilis* grown at 10 and 15°C exhibited lower growth densities (Fig. 4A). Depending on the culture temperature, the SGR is displayed in Fig. (4B). The rotifers cultured at 20°C had significantly higher SGR of 0.137±0.014 than those cultured at other temperatures.

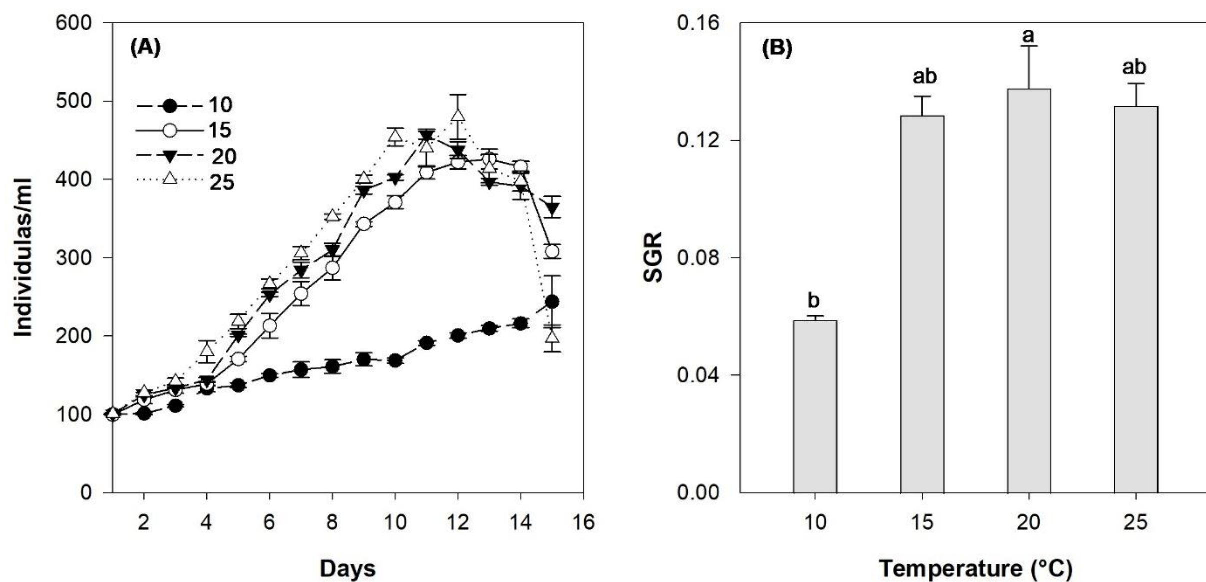

Fig. 4. Effects of water temperature on growth of *B. plicatilis*. (A) Growth density of *B. plicatilis* at different water temperatures. (B) Specific growth rate (SGR) of *B. plicatilis* at different water temperatures

Values are represented as mean ± standard deviation ($n = 3$). Different letters denote significant differences ($P < 0.05$).

At the end of this experiment, in the rotifers, the stored lipids stained with the Nile red demonstrated pronounced differences at different temperatures (Fig. 5). The amount of the stored lipids was more significant in the rotifers cultured at 10 and 15°C than those at 20 to 25°C.

2.2.Salinity

The investigation of the growth of the cultured rotifers in relation to salinity fluctuations at 20°C, which exhibited the highest SGR, showed that the maximum growth happened at 20psu on day 12, with 417±16.45 426 individuals/mL. The growth rate at a salinity level of 15psu was marginally greater than at a salinity level of 10psu, specifically from day 10 to day 13. The rotifers cultured at a salinity level of 30psu exhibited a significantly reduced growth rate, as shown in Fig. (6A). Data in Fig (6B)

illustrates that the highest SGR was at a salinity level of 20psu, with a value of 0.122±0.021. Conversely, the lowest SGR was recorded at a salinity level of 30psu, with a value of 0.067±0.002.

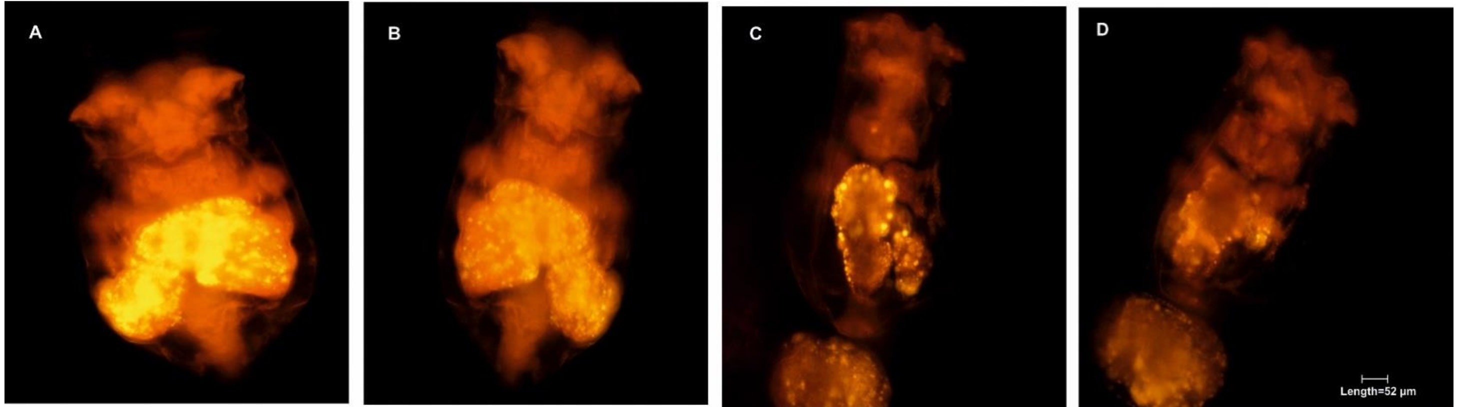

Fig. 5. Effects of water temperatures on the lipid content of *B. plicatilis* (A= 10, B = 15, C = 20, and D = 25°C), stained with Nile red

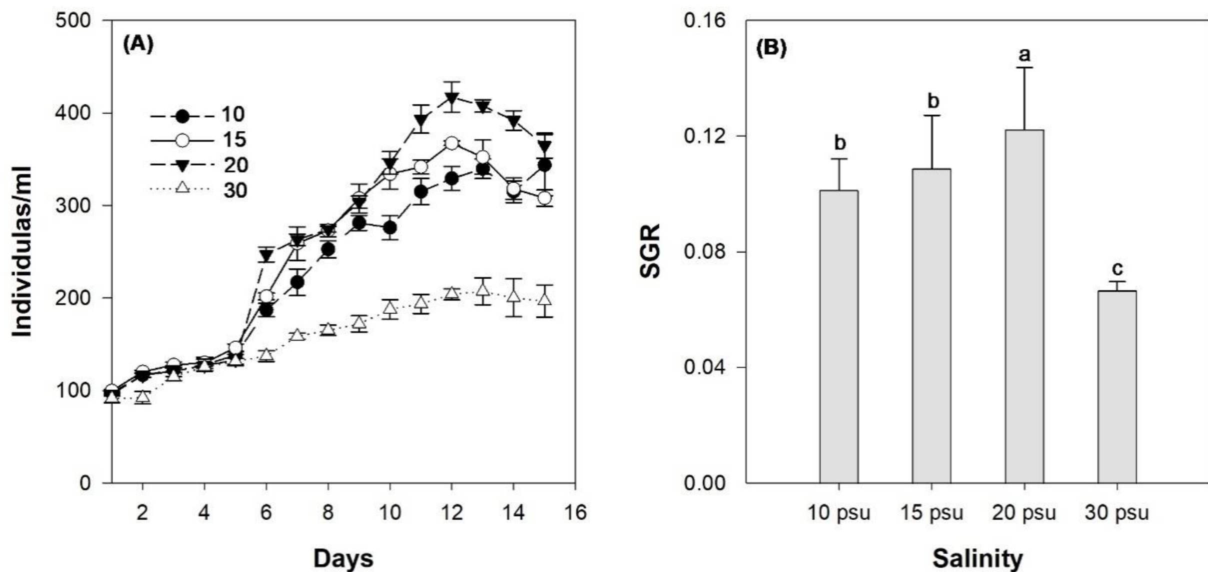

Fig. 6. Effects of water salinity on growth of *B. plicatilis*. (A) Growth density of *B. plicatilis* at different salinities; (B) Specific growth rate (SGR) of *B. plicatilis* at different salinities
Values are represented as mean ± standard deviation (n = 3). Different letters denote significant differences (P< 0.05).

3. Egg production rate

The highest egg production rate (EPR) of 2.31 and 1.75 eggs f/d (eggs per female per day) was recorded for the *Tetraselmis* sp. when 6×10^5 and 5×10^5 cells/mL were supplied, respectively. Females fed on the *Nannochloropsis* sp. showed their highest EPR of 1.12 eggs f/d at 6×10^5 cells/mL concentration, whereas for *Chaetoceros* sp., the highest EPR

of 0.49 eggs f/d was at 3×10^5 cells/mL concentration. The lowest EPR of less than 0.1 eggs f/d was recorded for both the *Nannochloropsis* sp. and *Chaetoceros* sp. treatments at the concentration of 2×10^4 cells/mL (Table 1). Significant differences in the *B. plicatilis* EPR were observed among different algal diets and concentrations ($P<0.01$), with a considerable increase in EPR with the increasing diet concentrations, except for the *Chaetoceros* sp. The total number of eggs produced by females demonstrated the same pattern of EPR; it seems that the productivity of the females was significantly different between algal diets and was usually higher at the highest food concentration (Table 1). When the *Tetraselmis* sp. and *Nannochloropsis* sp. were supplied at 6×10^5 cells/mL concentration, the highest number of eggs for each algal diet was produced, with 12.71 and 6.24 eggs/female, respectively.

The egg production rate of *B. plicatilis* was significantly different at various water temperatures (Fig. 7). The EPR of *B. plicatilis* ranged from 0.043 ± 0.0.07 to 2.5 ± 0.23 eggs f/d depending on the water temperature and was at its highest at 20°C and lowest at 5°C. An exceptionally high EPR of more than 2 eggs f/d was observed at 20– 25°C on days from 1 to 3; after that, it dropped to fewer than 1.5 eggs f/d. The EPR at 5, 10 and 30°C was lower than 1 egg f/d. However, at 15°C treatment, it was intermediate between the high and the low rates, with an EPR of 1.2–1.6 eggs f/d (Fig.7).

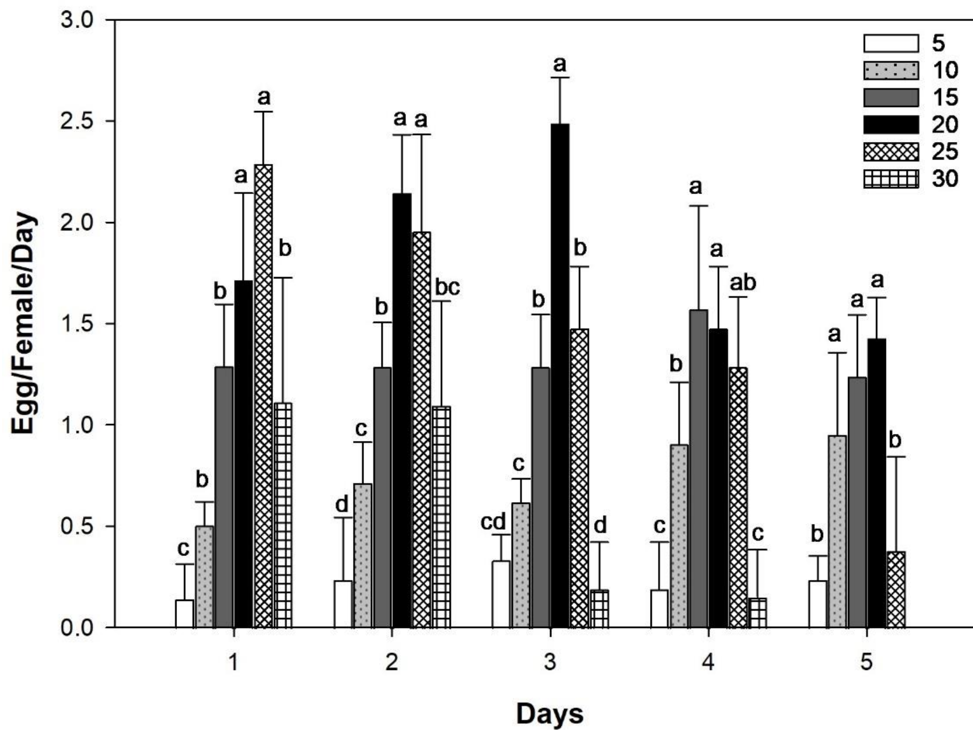

Fig. 7. Daily egg production rate (EPR) of *B. plicatilis* at different temperatures
Values are represented as mean ± standard deviation (n = 3). Different letters denote significant differences (P< 0.05).

Table 1: Egg production rate (EPR) of *B. plicatilis* with different types of algal diets and different concentration levels

Alagal diets concentrations	*Nannochloropsis* sp.		*Tetraselmis* sp.		*Chaetoceros* sp.	
	eggs f/d	**no.eggs/f**	**eggs f/d**	**no.eggs/f**	**eggs f/d**	**no.eggs/f**
2×10^4	0.05±0.04^{Cc}	0.50±0.14^{Bc}	0.25±0.08^{Ab}	2.50±0.57^{Ac}	0.06±0.06^{BCc}	0.60±0.14^{Bc}
1×10^5	0.10±0.01^{Cc}	0.70±0.23^{Cc}	0.31±0.03^{Abc}	3.10±0.28^{Ac}	0.17±0.14^{Bbc}	1.65±0.35^{Bbc}
2×10^5	0.15±0.06^{Cbc}	1.50±0.14^{Cbc}	0.41±0.06^{Ab}	4.10±0.14^{Abc}	0.27±0.18^{Bb}	2.65±0.49^{Bb}
3×10^5	0.24±0.08^{Cbc}	2.35±0.21^{Bb}	0.68±0.27^{Ab}	6.10±2.69^{Ab}	0.32±0.20^{Bab}	4.85±0.69^{ABa}
4×10^5	0.55±0.18^{Cb}	3.72±0.76^{Bb}	0.97±0.14^{Aab}	8.70±1.56^{Aab}	0.49±0.19^{Ba}	3.45±0.71^{Bab}
5×10^5	0.89±0.27^{Ba}	5.90±0.14^{Ba}	1.75±0.17^{Aa}	10.50±0.85^{Aa}	0.21±0.09^{Cbc}	2.10±0.51^{Cb}
6×10^5	1.12±0.12^{Ba}	6.24±0.91^{Ba}	2.31±0.13^{Aa}	12.71±1.06^{Aa}	0.12±0.07^{Cbc}	1.20±0.57^{Cbc}

All values are mean ± standard deviation(n = 3). The different capital letters indicate significant differences between algal diets, and different small letters indicate significant differences between concentrations (P< 0.05). eggs f/d = eggs per female per day, no.eggs/f= number of eggs per female.

DISCUSSION

The rotifers are generally non-selective feeder organisms that can consume various prey **(Yin & Zhao, 2008)**, mainly microalgae and diatoms **(Cruz-Cruz *et al.*, 2019)** by filter feeding, scraping, or browsing. Some rotifer species, such as *B. plicatilis*, are known to feed selectively **(Chotiyaputta & Hirayama, 1978)**. This rotifer species may be able to recognize their food particles and possess anatomical structures, such as cilia and mastax jaw, capable of accepting or rejecting particles **(Heerklob & Hlawa, 1995)**. The most widely used microalgae species in the mass culture of the rotifers in commercial aquaculture are the green algae of the genera *Nannochloropsis*, *Chlorella*, and *Tetraselmis*, which provide the rotifers with a high nutritional quality **(Bae & Hur, 2011; Abd Rahman *et al.*, 2018)**, in addition to some marine diatoms such as *Chaetoceros*.

An increasing interest has emerged in improving the nutrition of the rotifer *B. plicatilis* for its use as a part of live food in aquaculture and in the use of algae and diatoms endemic to the Nile Delta region, an area of major interest in promoting mariculture. This study examined the suitability of two species of green algae (*Nannochloropsis* sp. and *Tetraselmis* sp.) and the diatom *Chaetoceros* sp. as food sources for the rotifer *B. plicatilis* that was isolated from the Damietta estuary. We observed that the growth rate and reproductive capacity of *B. plicatilis* fed with *Tetraselmis* sp. was much higher than that of *B. plicatilis* fed with the other two algae. This may be due to the *Tetraselmis's* comparatively larger cell size and better nutritional quality than the other two algal species **(Alam & Shah, 2004)**. The optimal particle size range for *B. plicatilis* to consume is around 8 to less than 22 µm **(Hansen *et al.*, 1997)**. The efficiency of grazing reduces when the size of the food algae falls outside of this range **(Yin & Zhao, 2008)**. In the present study, *B. plicatilis* attained the highest SGR when fed with

Tetraselmis sp. (size = 10-12 μm), followed by *Nannochloropsis* sp. (size = 2-3 μm) and then the *Chaetoceros*(size = 4-6 μm). In addition to cell size, the distribution of algae in culture vessels is another characteristic of food quality **(Korstad *et al*., 1989)**. So, The higher reproductive rates of *B. plicatilis* with *Tetraselmis* can also be attributed to the availability of its cells to rotifers, as these flagellated motile algae are more equally dispersed in culture vessels than non-motile *Nannochloropsis* and *Chaetoceros*.

The food quantity also plays a role in the production efficiency of rotifers. Many studies **(e.g., Snell & Carrillo, 1984; Korstad *et al*., 1989; Hotos, 2002)** reported strong correlations between rotifer growth and food abundance. This study demonstrated that for each of the seven levels of food densities tested (i.e., 2×10^4, 1×10^5, 2×10^5, 3×10^5, 4×10^5, 5×10^5, and 6×10^5 cells /ml) for all algal species resulted in instant growth rates with good daily egg production, except at the lowest food density tested at 2×10^4 and 1×10^5 cells /ml for *Nannochloris* sp. This indicated that at low food concentrations, the types of food become a limiting factor **(Abd Rahman *et al*., 2018)**. The feature of *Tetraselmis* sp. mentioned previously gave them an advantage as the preferred rotifer food. The mean growth rates of rotifers increased significantly by increasing food densities from 2×10^4 cells /ml to 6×10^5 cells /ml, except for *Chaetoceros* sp. The low growth rates of *B. plicatilis* during the diatom feeding experiment, particularly at cell densities higher than 4×10^5 cells /ml, may reflect the negative Effect of *Chaetoceros* sp. on the survival of many rotifers. The diatom frustule may lead to incomplete digestion, followed by a low gut passage time of diatoms **(Ianora & Miralto, 2010)**. The irregular growth rate curves of rotifers fed *Chaetoceros* sp. compared to other algae, with high standard deviation values during the experiment and at most treatments, support the previous argument. Also, decreased rotifer productivity with high diatom densities could result from releasing toxic metabolites, such as antiproliferative compounds by *Chaetoceros,* when supplied as food of high densities **(Ianora, 2005)**.

In addition to the food algae, temperature and salinity are among the main factors influencing the reproductive rates in rotifers **(Yin & Zhao, 2008)**. The rotifers' responses to temperature and salinity changes, and food algae's responses depend on genotype **(Miracle & Serra, 1989)**. **Yufera (1987)** examined the effect of algal diet and temperature on productivity in two strains of *B. plicatilis*. In this investigation, it was observed that the rotifers fed *Nannochloropsis* exhibited the slowest rate, which closely resembled the rate observed in our isolated strain of *B. plicatilis*. On the other hand, **Korstad *et al*. (1989)** proposed that *Tetraselmis* cells exert an inhibitory influence on rotifers, with the slowest rates of growth observed in *B. plicatilis*-fed *Tetraselmis*. The findings of our investigation contrast with those of **Korstad *et al*. (1989).**

It has been shown that temperature positively affects both population productivity and the physiological rates in individual rotifers **(Miracle & Serra, 1989)**. This study observed that rotifers grown at 20 and 25°C had a higher growth rate than those cultured

at other temperatures. Previous studies have demonstrated that rotifers grow more slowly and take longer to reach maturity at lower water temperatures **(Yona, 2018; Yoo *et al.*, 2023)**. The highest density of rotifers cultured at 10°C was on day 15, approximately half their highest density at 25°C on day 10. **Lubzens and Minkoff (1988)** reported a maximum density within 5–8 days when rotifers are cultured at approximately 20-25 °C. However, the present study took approximately 10 days at 25 °C treatment, 11 days at 20°C, and 13 days at 15°C to reach the highest density. Different species strains may have maximum growth rates at different temperatures and periods **(Miracle & Serra, 1989)**. This may be attributed to (l) selection at the environmental temperatures of origin and (2) food interaction with temperature. In the present study, we used *Tetraselmis*at a 6×10^5 cells /ml density to feed rotifers in all treatments. This may ensure that the appropriate amounts of food in the culture medium meet the increased metabolic demands at elevated temperatures. Thus, the quiet difference between our study and those reported previously may be due to temperatures of origin during sampling of rotifers from the Damietta estuary since this was in spring at a water temperature of 21°C, making the isolated strain of *B. plicatilis* requires a longer time to reach its highest density.

The egg f/d at high temperatures was mainly higher than at low temperatures. According to **Dunca (1983)**, the temperature directly impacts the rate of egg development. The EPR has been used to predict the population growth in the culture. According to **Snell *et al.* (1987)**, the typical egg ratios of *B. plicatilis* cultured at 25°C ranged from 0.5- 1.2; once the egg ratio fell below 0.13, populations declined **(Fengqi, 1996)**. The results of this study demonstrated that, with the exception of 30°C, where the eggs f/d (0-1.11) were lower, the rotifers grown at high temperatures of 20–25 °C had higher eggs f/d (1.42-2.14 & 1.28-2.85, respectively). The isolated strain of *B. plicatilis* in the current study may belong to a distinct species that behaves differently from some other strains, as shown by the results of **Yona (2018)**, who found that the egg ratio was as high as 2.2 at 30°C. At high-temperature treatments, days one and two showed the highest values of eggs f/d, an then decreased until day 5. At the same time, at lower temperatures (10-15°C), a homogeneous increase from day one to day five was observed, resulting in a marginal increase in egg production over time. The rotifers cultured at 5°C had the lowest egg ratio (0.05-0.11) compared to other culture temperatures, indicating the significant effect of temperature on the *B. plicatilis* reproduction; the lower the temperature, the lower the productivity **(Yona, 2018)**.

Salinity is one of the most critical variables in controlling the dispersion of the rotifers **(Miracle *et al.*, 1987; Kaya *et al.*, 2010)**. Although the effect of salinity on the rotifer's survival and availability in the water column may be greater than temperature **(Fielder *et al.*, 2000)**, several rotifer genera such as *Brachionus* can tolerate salinities ranging from 1 to 97psu **(Walker, 1981)**. Generally, the availability of the rotifers decreased with significant changes in salinity from their optima **(Epp & Winston, 1977)**. The optimal salinity for the rotifers mass culture ranged from 10- 20psu**(Bosque *et al.*,**

2001; Yoo *et al*., 2023). In the present study, the rotifers growth was higher at 20 and 15psu than at 30psu. **Hong *et al.* (2024)** found that *B. plicatilis* requires more energy to reproduce in high-salinity conditions. Additionally, individuals die shortly after producing their last offspring, leading to a drop in the growth rate. These results indicate that the optimal salinity for the mass culture of *B. plicatilis* was between 15- 20psu.

The Nile red staining analysis was conducted to analyze the lipid storage at various food types and temperatures. According **to Lee *et al.* (2020)**, the Nile red-stained areas could indicate fatty acid storage in the rotifers. The sensitivity of the Nile red staining makes it a valuable method for comparing relative lipid levels in organisms, even if the lipids levels are below the detection limits of some analytical techniques **(Carman *et al*., 1991)**. The large stained areas in *B. plicatilis* fed the *Tetraselmis* sp. compared to other algae may be due to the accumulation of the polyunsaturated fatty acids that account for 94.7% of the total lipids **(Khairy & El-Sayed, 2012; Ali, 2020)**. At lower temperatures of 10 and 15°C, the stained areas were comparatively more prominent than those at higher temperatures (20 and 25°C), indicating the accumulation of fatty acids at lower temperatures. Several studies have revealed that aquatic invertebrates try to increase the fatty acid composition at low temperatures. For example, the cyclopoid copepod *Paracyclopina nana* responded to lower temperatures by increasing its n-3 fatty acid content **(Lee *et al*., 2017)**. Moreover, the plankton in the polar region store about 80% of total carbon as lipids, whereas the plankton in the warm water store less than 20% **(Smith & Morris, 1980)**.

CONCLUSION

The available information in the present study and/or other studies on the effects of different microalgae diets on the *B. plicatilis* productivity indicates that the suitability of a particular algal species in the rotifer rearing depends on various factors such as nutritional quality, algal size, and algal density. Our results indicat that the *Tetraselmis* sp. was the best diet, enhancing the egg production and growth rate of the rotifers, followed by *Nannochloropsis* sp.; the productivity of the rotifers increased with increasing food densities for the previous two algal species but not for *Chaetoceros* sp. This study also reveals that temperature and salinity affect the population growth of *B. plicatilis* significantly. The optimal temperature was 20°C, and the optimal salinity was approximately 15 to 20psu; a high salinity of more than 30psu reduced the rotifer's productivity. Low temperatures delay egg production, while higher temperatures of less than 30°C result in faster production with a high egg ratio.

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