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Original Article

Effects of cultural conditions on life history characteristics of the freshwater rotifer *Brachionus calyciflorus*

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Abstract: The effects of temperature, food concentration, and pH conditions on the life history characteristics of the freshwater rotifer, *Brachionus calyciflorus*, were investigated. The culture temperature (20, 25, and 30°C) had a significant relationship with life history parameters. At lower temperatures, there was a prolongation of the mean lifespan and juvenile period. The optimal temperature for the fecundity of this species was 25°C with an average quantity of 23.67±6.99 offspring female^{-1} across the entire lifespan of 6.7 days. No significant difference was found between the mean lifespan at different algae densities but the maximum fecundity (25.75±6.99 offspring female^{-1}) was obtained at an algae density of 10x10^{6} cells.ml^{-1}. *Brachionus calyciflorus* could tolerate a broad range of pH (4-10) but preferred pH from 6-10. These results are critical for potential applications of this species in ecotoxicology, biomonitoring as well as in mass culture as live food for larval rearing in aquaculture.

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Introduction

Rotifers are aquatic invertebrates that occur in almost all types of water bodies worldwide. Rotifers are critical linkages between phytoplankton and planktivorous fish (Wallace et al., 2006). They are a valuable live food source for the larvae culture of many fish species (Lubzens, 1987). The rotifers were represented by many species of *Brachionus* and others, indicating the trophic status of water bodies (Baruah et al., 1996; Nogueira, 2001; Ismail and Adnan, 2016).

Among freshwater monogonont rotifers, *Brachionus calyciflorus* Pallas, 1766 is widely studied in many fields, including ecology (Gilbert, 1985; Guo et al., 2011), evolutionary biology (Becks and Agrawal, 2012; Scheuerl and Stelzer, 2013; Declerck et al., 2015), ecotoxicology (Snell and Moffat, 1992; Cruciani et al., 2016; Han et al., 2018), and aquaculture (Lim and Wong, 1997). *Brachionus calyciflorus* was also the first monogonont rotifer that its genome was characterized (Kim et al., 2018) and suggested to be a species complex with at least four cryptic species (Papakostas et al., 2016). In Vietnam, most studies on rotifers focused on their biodiversity and distribution (Shirota, 1966; Dang and Ho, 2002; Zhdanova, 2011; Phan and Le, 2012; Trinh-Dang et al., 2015, 2019; Duong-Quang et al., 2020). Some species of the genus *Brachionus* such as *B. plicatilis, B. rotundiformis, B. angularis,* and *B.* have been investigated to be used as live food for aquaculture in laboratory conditions (Le et al., 2017; Quy et al., 2018; Cong et al., 2019; Vu et al., 2021).

Accurate estimation of the biological characteristics of rotifers in the field is quite difficult. Meanwhile, laboratory culture of organisms under similar natural ranges of environmental parameters is one of the methods to determine growth and reproductive parameters. In addition, life history characteristics such as lifespan and fecundity are important approaches to studying the life history

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strategy and population dynamics of zooplankton under continuously changing environmental conditions (Wallace et al., 2006). In the present study, *B. calyciflorus* was collected from a freshwater body in Danang city, Central Vietnam, an area with a tropical monsoon climate, and cultured under laboratory conditions. Then, its juvenile period, embryonic development, spawning interval, fecundity, and mean lifespan were determined under different culture conditions of temperature, food concentration, and pH.

Materials and methods

Sample collection and stock culture: *Brachionus calyciflorus* individuals were isolated in December 2020 from Lake Cong Vien 29/3 (16°03'51.8"N 108°12'15.6"E) in Danang city, Vietnam, and then cultured under laboratory conditions at the Laboratory of Algal Technology, Faculty of Biology and Environmental Science, The University of Danang, Vietnam. Stock cultures were kept under static-renewal conditions for approximately 3 months with fluorescent light at 25±1°C in an EPA medium. The EPA medium is prepared by dissolving 96 mg of $NaHCO_3$, 60 mg of $CaSO_4$, 60 mg of $MgSO_4$, and 4 mg of KCl in 1 L of distilled water (Peltier and Weber, 1985). Rotifers were fed every 2 days with the green algae of *Chlorella vulgaris* at a density of $1.0\text{-}2.0 \times 10^6$ cells.ml^{-1}, which was semi-continuously cultured in BBM medium with 16: 8 light: dark photoperiod of 3000 lx fluorescent light. Before feeding the rotifers, the algae were precipitated by centrifugation (3000 rpm for 5 minutes), resuspended in an EPA medium, and then stored at 4°C. The density of the stock algae was estimated using a hemocytometer (Tiefe, 0.1 mm, 1/400 qmm, Germany).

Life history assays: Life history experiments were conducted in 96-well tissue cultures plates and started by introducing amictic neonate (< 2 h old) into each well containing 0.3 ml of EPA medium. A total of 52 individuals were included and set up at three temperature levels, six pH levels, and four food concentrations. Four replicates were made for each setup of the experiment.

Specifically, to examine the effects of different temperatures on the life cycle of *B. calyciflorus*, the solutions were maintained at different temperatures (20, 25, and 30°C) and fed on the algae *C. vulgaris* at 10×10^6 cells.ml^{-1}. For the impact of different food concentrations, the experiments were implemented with solutions at different algae densities, including *C. vulgaris* at 1.5, 5, 10, and 15×10^6 cells.ml^{-1} at 25°C. For pH experiments, the solutions with the required pH (3.5, 4, 6, 7, 8, and 10) were maintained at 25°C and an algae density of 10×10^6 cells. ml^{-1}. The pH of the culture medium was measured with a pH meter (Hach HQ411D) and adjusted by adding NaOH (0.1 mol. L^{-1}) and HCl (0.1 mol.L^{-1}) into the medium (Mitchell, 1992).

The rotifers were observed every 1.5 h with a stereo microscope. The time of the first egg, the number of eggs and neonates produced, and the number of original individuals alive were recorded, respectively, and the neonates were removed. Before each individual of every cohort died in the life history experiments, the original rotifers were transferred into a freshly prepared test solution every 24 h. Based on the data collected, five life history parameters were calculated based on previously published methods, including the juvenile period (the time between a neonate and that laying the first egg), embryonic development (the time between an adult laying an egg and the egg hatching), spawning interval (the time between two spawnings), fecundity (the number of offspring produced per female), mean lifespan (the average surviving time of all females) (Paez, 1991; Walz, 1983).

Data analysis: Differences between means for each life history parameter were compared using a one-way analysis of variance (ANOVA). Significant differences ($P<0.05$) were further analyzed with Tukey's Test. All statistics were calculated using the R program (R Core Team, 2020).

Results

Morphological characteristics: Some morphological characteristics at different stages of the life cycle

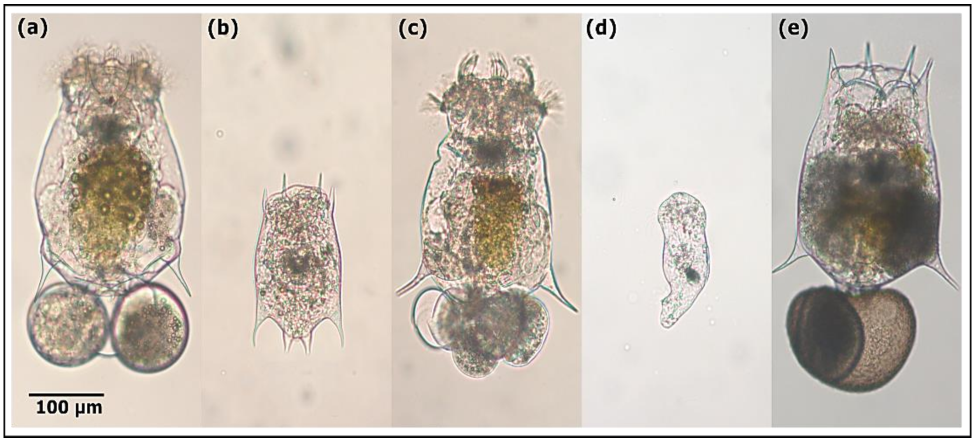

Figure 1. Morphological characteristics of life-cycle stages of *Brachionus calyciflorus* isolated from Danang, Central Vietnam. (a) Amictic female carrying three diploid eggs developing parthenogenetically into females; (b) Newborn female; (c) Mictic female carrying five haploid eggs developing parthenogenetically into haploid males; (d) Newborn male; (e) Fertilized mictic female carrying one encysted diapausing embryo, or resting egg.

Table 1. Morphological characteristics of life-cycle stages of *B. calyciflorus* isolated from Danang, Central Vietnam.

Life-cycle stages of *B. calyciflorus*	Length (µm)	Width (µm)	Posterior lateral spines (µm)	Anterior lateral spines (µm)	Anterior median spines (µm)
Adult female	298.00±23.07	192.67±11.68	60.00±2.65	43.00±2.00	52.33±2.52
Newborn female	219.33±10.07	119.67±10.02	44.67±1.53	28.33±1.53	32.67±2.08
Newborn male	165.67±4.04	60.67±2.08	-	-	-
Diploid egg	112.67±5.69	102.00±6.24	-	-	-
Haploid egg	86.67±2.08	62.33±2.52	-	-	-
Resting egg	182.67±6.43	120.33±5.51	-	-	-

Table 2. Juvenile period (JP), embryonic development (ED), spawning internal (SI), fecundity (F), mean lifespan (ML) of *Brachionus calyciflorus* at different temperatures (mean±SD). Within a column, means with the same superscript letter are not significantly different (P>0.05).

Temperatures (°C)	JP (h)	ED (h)	SI (h)	F (offspring. female^{-1})	ML (h)
20	33.00±1.00^{a}	21.00±6.00^{a}	6.11±0.51^{a}	11.00±8.19^{a}	218.67±14.43^{a}
25	19.50±1.29^{b}	11.50±0.58^{b}	3.96±0.93^{b}	23.67±6.99^{b}	161.25±29.61^{b}
30	9.00±1.00^{c}	9.33±0.58^{b}	2.22±0.38^{c}	13.67±1.53^{ab}	64.33±10.97^{c}

of *B. calyciflorus* isolated from Danang, Central Vietnam are shown in Figure 1. The average body length and width (w) of adult females (mean±standard deviation, n=3) were 298.00±23.07 and 192.67±11.68 µm, respectively. Newborn males and females produced by adult females had smaller body lengths, which were 165.67±4.04 and 219.33±10.07 µm, respectively. In addition, females of *B. calyciflorus* produced three different types of egg viz. diploid, haploid, and resting with average lengths of 112.67±5.69, 86.67±2.08, and 182.67± 6.43 µm, respectively (Table 1).

Effects of temperature on life-history parameters of *B. calyciflorus*: The environmental temperature had a major effect on the life-history parameters of *B. calyciflorus*. The juvenile period, embryonic development, spawning internal, and mean lifespan steadily decreased with the increase of the environmental temperature (Table 2, Fig. 2). In particular, the mean juvenile period of *B. calyciflorus* was 33±1, 19.5±1.29, and 9±1 h at 20, 25, and 30°C, respectively (P<0.001). At the higher temperature, the mean lifespan was also significantly reduced (P<0.05). Nevertheless, the optimal temperature for the fecundity of this species was at 25°C with an average quantity of 23.67±6.99 offspring female^{-1} across the entire lifespan of 6.7 days. At this temperature, the highest quantity of 35

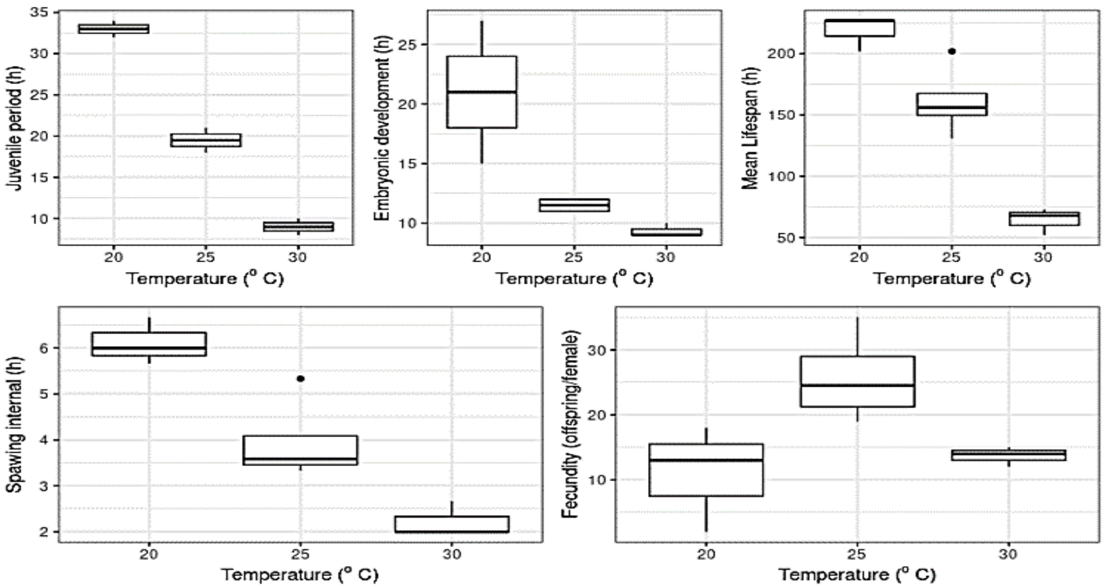

Figure 2. Life history characteristics of *Brachionus calyciflorus* at different temperatures.

Table 3. Juvenile period (JP), embryonic development (ED), spawning internal (SI), fecundity (F), mean lifespan (ML) of *Brachionus calyciflorus* at different algae densities (mean±SD). Within a column, means with the same superscript letter are not significantly different (P>0.05).

Densities of *C. vulgaris* (cells.mL^{-1})	JP (h)	ED (h)	SI (h)	F (offsprings. female^{-1})	ML (h)
1.5 x 10^6	25.33±6.03^{a}	11.00±1.00^{a}	15.78±5.83^{a}	7.67±2.31^{a}	177.33±61.01^{a}
5 x 10^6	21.33±4.04^{a}	11.00±1.73^{a}	5.81±1.89^{b}	14.33±6.81^{ab}	155.33±24.01^{a}
10 x 10^6	19.5±1.29^{a}	11.50±0.58^{a}	3.96±0.93^{b}	25.75±6.99^{b}	161.25±29.61^{a}
15 x 10^6	18.50±0.58^{a}	12.00±0.82^{a}	3.25±0.69^{b}	23.25±4.65^{b}	176.75±25.75^{a}

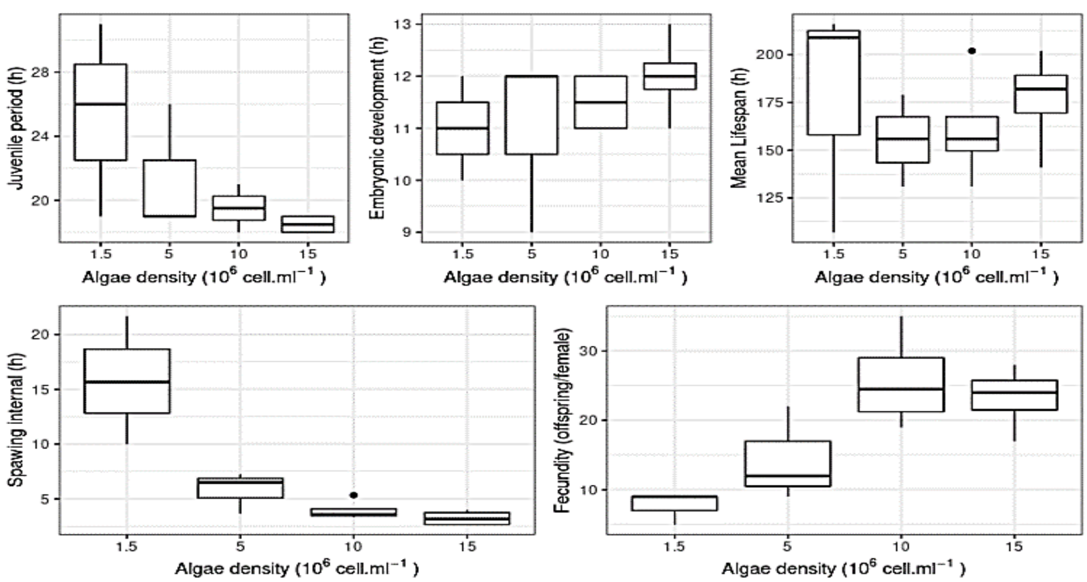

Figure 3. Life history characteristics of *Brachionus calyciflorus* at different densities of algae *C. vulgaris*.

offspring female^{-1} was also reached. At 30°C, the mean fecundity of individuals was slightly higher than those maintained at 20°C with values of 13.67±1.53 and 11.00±8.19 offspring female^{-1}, respectively.

Effects of algae density on life history parameters of *B. calyciflorus*: The life history parameters of *B. calyciflorus* at different densities of *C. vulgaris* as live food for the rotifers are shown in Table 3. The juvenile period and spawning internal of this species

Table 4. Juvenile period (JP), Embryonic development (ED), Spawning internal (SI), Fecundity (F), Mean lifespan (ML) of *Brachionus calyciflorus* at different pH levels (mean±SD). Within a column, means with the same superscript letter are not significantly different (P>0.05).

pH	JP (h)	ED (h)	SI (h)	F (offsprings. female^{-1})	ML (h)
3.5	All individuals could not survive to 24h				
4	22.50 ± 4.12^{a}	10.25 ± 1.50^{a}	8.00 ± 2.76^{a}	12.50 ± 4.73^{a}	158.00 ± 34.13^{a}
6	20.00 ± 1.00^{a}	12.00 ± 1.00^{ab}	3.22 ± 0.38^{b}	23.00 ± 6.00^{a}	168.00 ± 0.00^{a}
7	18.25 ± 0.5^{a}	13.75 ± 0.50^{b}	3.29 ± 1.39^{b}	24.50 ± 6.45^{a}	179.00 ± 0.00^{a}
8	19.33 ± 1.53^{a}	11.67 ± 1.15^{ab}	4.17 ± 0.83^{ab}	23.67 ± 2.52^{a}	183.00 ± 17.35^{a}
10	19.50 ± 1.91^{a}	12.00 ± 0.82^{ab}	4.71 ± 1.69^{ab}	20.75 ± 5.91^{a}	173.25 ± 22.02^{a}

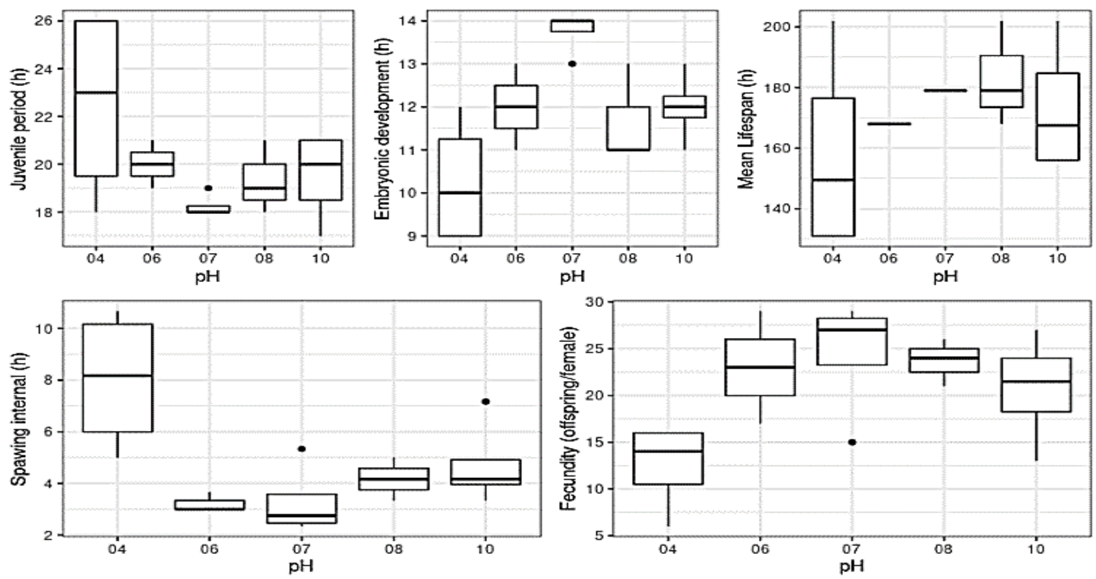

Figure 4. Life history characteristics of *Brachionus calyciflorus* at different pH levels.

were decreased with increasing food concentration (Fig. 3). The mean juvenile period decreased from 25.33±6.03 h at an algae density of 1.5x10^{6} cells.ml^{-1} to 18.50±0.58 h at a density of 15x10^{6} cells.ml^{-1}. Besides, a significant difference was also observed in the spawning internal between the algae density of 1.5x10^{6} cells.ml^{-1} and the higher experimental algae densities (P<0.01).

The maximum fecundity of *B. calyciflorus* was 25.75±6.99 offspring female^{-1} at a density of 10x10^{6} cells.ml^{-1} and the minimum fecundity of 7.67±2.31 offspring female^{-1} was found at a density of 1.5x10^{6} cells.ml^{-1} (3.4 times smaller). No significant difference was found between the fecundity at densities of 10x10^{6} and 15x10^{6} cells.ml^{-1}, which showed that the number of offspring by females could reach a limited value in the experiment.

For lifespan, no significant difference was found between different algae densities. The lowest mean lifespan (155.33±24.01 h) was observed at a density of 5x10^{6} cells.ml^{-1}. The highest mean lifespan (177.33±61.01 h) was found at a density of 1.5x10^{6} cells.ml^{-1}, however, the values of individuals were unstable. At densities of 10x10^{6} and 15x10^{6} cells.ml^{-1}, the mean lifespans were 161.25±29.61 and 176.75±25.75 h, respectively.

Effects of pH level on life history parameters of *B. calyciflorus*: *Brachionus calyciflorus* could tolerate a broad range of pH from 4 to 10 in the experimental condition (Table 4, Fig. 3). However, this species prefers a pH of 6-8 and might adapt to alkaline environments rather than acidic environments. This is demonstrated by a better performance of most of the life history parameters at pH from 6 to 10. In contrast, the instability of these parameters was observed at pH 4, and all individuals of this species could not survive to 24h at pH 3.5. The embryonic development and spawning internal

Table 5. Morphological characteristics of *Brachionus calyciflorus* strains from water bodies.

Life-cycle stages of *B. calyciflorus*	Length (µm)	Width (µm)	Posterior lateral spines (µm)	Anterior lateral spines (µm)	Anterior median spines (µm)	Origin	References
Adult female	298.0±23.1	192.7±11.6	60.0±2.7	43.0±2.0	52.3±2.5	Da Nang, Vietnam	This study
	291±24	193±16	-	-	-	Florida, USA	Lim et al. (1997)
	244.0±21.1	160.7±18.6	-	-	-	Gainesville, USA	Rico-Martínez et al. (1992)
	277.3±23.0	164.3±16.8	-	-	-	Tampa, USA	
	195.3±16.8	121.3±18.1	-	-	-	McFarland, USA	
	266.0±21.9	163.0±25.2	-	-	-	Madison, USA	
	249.6±1.2	158.5±0.7	44.3±1.3	35.4±0.5	46.3±0.5	Lake Fengming, China	Xue et al. (2017)
	239.8±1.6	153.2±0.9	33.0±1.2	33.0±0.5	48.2±0.7	Lake Hui, China	
	255.1±1.4	160.6±0.8	34.5±1.0	36.0±0.5	51.1±0.6	Lake Tingtang, China	
	196.9±14.6	-	-	-	-	A pond in Guangzhou, China	Xi et al. (2002)
	176.1±23.5	-	-	-	-	A pond in Wuhu, China	
	196 ± 12	156 ± 8	-	-	-	A pond in Beijing, China	Yin et al. (2008)
Diploid egg	112.7±5.7	102.0±6.2	-	-	-	Da Nang, Vietnam	This study
	122.6±0.7	87.1±0.3	-	-	-	Lake Fengming, China	Xue et al. (2017)
	116.7±0.9	84.8±0.5	-	-	-	Lake Hui, China	
	116.3±0.8	82.6±0.5	-	-	-	Lake Tingtang, China	

of this species had a significant difference between pH 7 and pH 4 (P<0.05).

Discussion

Differences in morphological characteristics between *B. calyciflorus* strains: In this study, *B. calyciflorus* was found in both male and female forms, and also resting eggs were observed. Most planktonic rotifers have a cyclical parthenogenetic life cycle where asexual reproduction predominates, but there are periods where both asexual and sexual reproduction occur simultaneously (Snell and Carmona, 1995). In monogonent rotifers, asexual reproduction in the absence of males (amictic phase) is mixed with occasional bouts of sexual reproduction (mictic phase). Asexual (amictic) females are diploid, they produce eggs mitotically that develop into females (Birky Jr and Gilbert, 1971). Asexual females after receiving the mimic stimulus can produce both sexual (mictic) and asexual daughters. Sexual female production is then followed by male production, fertilization, and resting egg formation. Sexual females produce haploid eggs which can develop into haploid males if unfertilized. In contrast, if fertilization happens, mictic eggs become diploid and develop into resting eggs (Gilbert, 1974). The resting eggs after a period of dormancy will hatch into asexual females when receiving specific cues and enter into the asexual phase again (Pourriot and Snell, 1983).

There were significant differences in morphological characteristics and life table parameters between rotifer species or strains (Wang et al., 2014). In this study, the morphological characteristics of the life-cycle stages of *B. calyciflorus* isolated from Danang, Central Vietnam were investigated and the body size of adult females and diploid egg size were measured and compared with other strains from various water bodies (Table 5). In general, the adult females of *B. calyciflorus* had body length ranges from 176 to 298 µm and body width ranges from 121 to 193 µm. *Brachionus calyciflorus* isolated from Danang is one of the largest known body lengths of adult females and is much larger than other strains from several water bodies around the world (Rico-Martínez et al.,

Table 6. The effects of temperature conditions on life history characteristics of *Brachionus calyciflorus* strains from water bodies.

Temperatures (°C)	JP (h)	ED (h)	F (offspring. female^{-1})	ML (h)	Origin	References
15-16	39.8±6.2	-	14.1±4.7	270.9±71.5	A pond in Gainesville, Florida	Kauler et al. (2011).
	45.7±1.2	29.4±1.5	-	334.2±8.3	Lake Baixiang, China	Wang et al. (2014)
	43.4±1.2	25.1±1.1	-	212.6±10.3	Lake Kongque, China	
20-22	33.0±1.0	21.0±6.0	11.0±8.2	218.7±14.4	Da Nang, Vietnam	This study
	24.6±2.2	13.4±1.7	-	-	Ghent, Belgium	Awaïss et al. (1992)
	31.8±1.1	17.1±1.0	13.0 ± 0.29	154.4 ± 16.1	Lake Dianchi, China	Xiang et al. (2016)
	31.9±0.8	16.5±0.2	11.3 ± 0.23	167.2 ± 1.6	Xishuangbanna, China	
	18 ± 4.3	-	14.7±5.1	153.1±44.4	A pond in Gainesville, Florida	Kauler et al. (2011)
	36.2±0.9	21.4±1.1	-	340.8±8.5	Lake Baixiang, China	Wang et al. (2014)
	28.4±0.1	19.7±0.8	-	176.4±4.9	Lake Kongque, China	
24-25	19.5± 1.3	11.5± 0.6	23.7± 6.9	161.3±29.6	Da Nang, Vietnam	This study
	17.9±0.8	10.1±1.2	-	-	Ghent, Belgium	Awaïss et al. (1992)
	32.4±0.7	17.7 ± 0.8	13.2 ± 0.6	146.4 ± 7.7	Lake Dianchi, China	Xiang et al. (2016)
	17.9±1.1	17.9 ± 0.3	14.6 ± 0.9	166.4 ± 8.1	Xishuangbanna, China	
	25.7±0.6	12.6±0.4	-	208.9±7.7	Lake Baixiang, China	Wang et al. (2014)
	17.8±0.3	15.6±0.5	-	95.6±2.6	Lake Kongque, China	
28-32	9.0±1.0	9.3±0.6	13.7±1.5	64.3±10.9	Lake Cong vien 29/3, Vietnam	This study
	10.8±1.2	7.0±0.9	-	-	Ghent, Belgium	Awaïss et al. (1992)
	16.3±1.6	11.3±0.6	11.5 ± 0.6	48.8 ± 5.8	Lake Dianchi, China	Xiang et al. (2016)
	16.5±0.7	10.0±0.4	13.0±1.3	73.6±3.5	Xishuangbanna, China	
	7.7±2.4	-	17.3±6.1	98.9±27.1	A pond in Gainesville, Florida	Kauler et al. (2011).
	15.8±0.5	12.0±0.5	-	161.2±4.6	Lake Baixiang, China	Wang et al. (2014)
	13.9±0.3	9.3±0.4	-	79.3±2.0	Lake Kongque, Chin	

1992; Xi et al., 2002; Yin et al., 2008). Some variation exists in size and growth rate among different strains of *B. calyciflorus* are similar to the variation found for *B. plicatilis* (Snell and Carrillo, 1984). Consequently, the variation among strains gives this species an advantage over other species in its use as food for larval fish because different size strains will be suitable for different-sized larval fish (Rico-Martínez and Dodson, 1992). In contrast, there were not many differences in the diploid egg size of *B. calyciflorus* among strains, which range from 112.67 to 122.6 μm in length and from 82 to 102 in width.

Effects of temperature, food concentration, and pH conditions on life history parameters of *B. calyciflorus*: Various factors, including temperature, food concentration, and pH conditions affect the survival and reproduction of rotifers. Temperature is one of the factors that most influence the population growth of rotifers. Previous works indicated that the responses in life-history traits to increasing temperature differed not only for different species of rotifers but also for different strains of the same species (Awaïss et al., 1992; Kauler et al., 2011; Wang et al., 2014; Xiang et al., 2016). In general, the developmental rate of poikilothermic animals depends on the metabolic rate, which increases with temperature. Therefore, increasing temperature will lead to a decrease in life expectancy at hatching, average lifespan, and generation time of rotifers (Xiang et al., 2010). In the present study, we found that the juvenile period, embryonic development, spawning internal, and mean lifespan were steadily decreased with increasing environmental temperature, but the optimal temperature for the fecundity of this species was

Table 7. The effects of food concentrations on life history characteristics of *Brachionus calyciflorus* strains from water bodies.

Concentrations of algae ($cells.ml^{-1}$)		JP (h)	ED (h)	F ($offsprings.female^{-1}$)	ML (h)	Origin	References
Chlorella vulgaris	$1.5x10^6$	25.3±6.0	11.0±1.0	7.7±2.3	177.3±61.0	Da Nang, Vietnam	This study
	$5x10^6$	21.3±4.0	11.0±1.7	14.3±6.8	155.3±24.0		
	$10x10^6$	19.5±1.3	11.5±0.6	25.75±7	161.3±29.6		
	$15x10^6$	18.5±0.6	12.0±0.8	23.3±4.7	176.8±25.8		
Scenedesmus obliquus	$0.5x10^6$	37.1±1.2	14.5±0.9	6.7±0.4	201.0±4.2	Lake Baixiang, China	Wang et al. (2014)
	$1.0x10^6$	30.4±1.3	13.3±0.6	6.4±0.5	203.3±5.8		
	$2x10^6$	28.5±0.8	13.4±0.9	7.7±0.5	203.1±6.1		
	$4x10^6$	25.7±0.6	12.6±0.4	10.7±1.11	208.9±7.7		
Scenedesmus obliquus	$2x10^6$	32.4±0.7	17.7±0.8	13.2±0.6	146.4±7.7	Lake Dianchi, China	Xiang et al. (2016)
	$2x10^6$	17.9±1.1	17.9±0.3	14.6±0.9	166.4±8.1	Xishuangbanna, China	
Scenedesmus obliquus	$3x10^6$	-	-	6.4±0.4	91.6±7.8	Lake Liantang, China	Xiang et al. (2010)
Chlorella pyrenoidosa	$5x10^6$	20.4±2.3	-	5.8±1.4	72.2±10.4	Lake Donghu, China	Xi et al. (2001)

25°C. The effects of temperature on the net reproductive rate and fecundity differ not only among different rotifer strains but also among distant morphotypes (Xiang et al., 2010; Wang et al., 2014). Xiang et al. (2010) suggested that the net reproductive rate of the two-spined *B. calyciflorus* was higher at 30°C than at the other temperatures, but the net reproductive rate of the unspined rotifer was not affected by temperatures.

Algae concentration is one of the important factors to influence the growth, movement, and reproduction of rotifers. In theory, a high algae concentration in the environment will increase the opportunity for rotifers to be fed enough, thus, might grow well (Liang et al., 2017). Aside from algal concentration, algal diets and algal food quality were also reported to affect the life history of amictic rotifers (Ruttner-Kolisko, 1984; Jensen and Verschoor, 2004). According to Xi et al. (2001), there were no significant effects of algal food type on the duration of the reproductive period of the three types of females, but a significant effect on the duration of the juvenile period of amictic females and unfertilized mictic females was recorded. The duration of the juvenile period of amictic females fed *C. pyrenoidosa* was shorter than that of those fed *S. obliquus* or a mixture of both algae species, and that of unfertilized mictic females fed *C. pyrenoidosa* was longer than that of those fed *S. obliquus*. Thus, these two types of females appear to respond differently to these algae.

Rico-Martínez and Dodson (1992) suggested that the optimum conditions for raising the rotifer *B. calyciflorus* were a temperature of 30°C and a food concentration of 10^7 $cells.ml^{-1}$ of the algae *C. vulgaris*. This is confirmed by our results as we explored the maximum fecundity of *B. calyciflorus* at the algae concentration of $10x10^6$ $cells.ml^{-1}$ and no significant difference was found between the fecundity of this species at concentrations of $10x10^6$ $cells.ml^{-1}$ and $15x10^6$ $cells.ml^{-1}$. Nevertheless, the appropriate algae concentration for raising the rotifer in general also depends on temperature. At a temperature of 20°C and high food concentration of $5x10^7$ $cells.ml^{-1}$, negative growth rates were found. This result was in line with other studies (Halbach and Halbach-Keup, 1974; Neimeroth, 1980), which explain the negative growth as being a consequence of high respiratory costs.

The response of *B. calyciflorus* to pH also received many concerns (Mitchell and Joubert, 1986; Mitchell, 1992; Yin and Niu, 2008). The distribution and abundance of rotifers are confirmed to be affected by pH (Wallace and Snell, 2001). Species in the genus *Brachionus* such as *B. angularis*, *B. calyciflorus*, and *B. quadridentatus* are believed to be common alkaline species. *B. calyciflorus, B. quadridentatus, B. urceolaris,* and *B. patulus* showed a higher fecundity at pH from 6 to 8 (Yin and Niu, 2008). Mitchell (1992) found that the organisms were unable to survive for 24 h at a pH of 11.5, and the lethal concentration of the organisms was less than 2 days at a pH of 2.5. Similar results were obtained in this study as we recorded *B. calyciflorus* prefers a pH of 6-10 and might adapt to alkaline environments rather than acidic environments. This is demonstrated by a better performance of most of the life history parameters at pH from 6 to 10. In contrast, the instability of these parameters was observed at pH 4. All individuals of this species could not survive for 24 hours at pH 3.5.

Conclusion

Ambient temperature, pH, and food concentration are important factors to influence the rotifer *B. calyciflorus* isolated from the freshwater body in Vietnam. The optimal temperature for the fecundity of this species was 25°C and a food concentration of 10×10^6 cells.ml^{-1} of *C. vulgaris*. Similar to other species and strains in the genus Brachionus, the species *B. calyciflorus* in our study also prefer alkaline environments than acidic environments, which pH ranges from 6 to 10.

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