

Effect of diet and temperature on the culture of *Alona guttata* (Sars, 1862) (Cladocera: Chydoridae) under laboratory conditions

Efecto de la dieta y temperatura en el cultivo de *Alona guttata* (Sars, 1862) (Cladocera: Chydoridae) en condiciones de laboratorio

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ABSTRACT

Background. Chydoridae is the most diverse family of cladocerans but information about their biology or life cycles is still limited, perhaps because of the hard task that their taxonomy involves or that culture in optimal conditions are not described for several species. **Goals.** This study examined the effects of culture conditions (algal concentration, algal species, and temperature) on the demography of *Alona guttata* (Sars, 1862) to obtain their maximal growth rates. **Methods.** Life table analysis with the chydorid *A. guttata* were performed as follows: five females per cohort (six replicas) were fed on either *Chlorella vulgaris* (Beijerinck, 1890) or *Nannochloropsis oculata* (Hibberd, 1981) at 0.5×10^6 or 2×10^6 cells/mL, reared at 20°C or 25°C and photoperiod 16:8 h (light: dark). Media was supplemented with an artificial substrate. Then, daily fertility and survival were assessed to estimate the demographic parameters: average lifespan (ALS), life expectancy at birth (LEB), generation time (GT), gross reproductive rate (GRR), longevity, net reproductive rate (NRR), and the intrinsic rate of population increase (r). **Results.** Significant effects were observed due to the three factors tested and their interactions. Increasing algal concentrations of either *C. vulgaris* or *N. oculata* promoted higher fertility and longer survival. Lower temperature extended the ALS and LEB when organisms were fed on the highest algal concentration. The highest r values were observed when *Alona* was fed on *N. oculata* at 2×10^6 cells/mL. **Conclusions.** The best culture conditions, in terms of the population growth rates of *A. guttata*, were provided by *N. oculata* at 2×10^6 cells/mL at 25°C with the supplementation of artificial substrate.

Keywords. Anomopoda, demographic parameters, fertility, life table analysis, survival

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RESUMEN

Antecedentes. La familia Chydoridae es la más diversa de los cladóceros pero la información acerca de su biología o sus ciclos de vida es aún limitada, posiblemente por la dificultad que representa su taxonomía o porque las condiciones óptimas de cultivo no han sido descritas para varias especies. **Objetivos.** Este estudio examina los efectos de las condiciones de cultivo (concentración de algas, especie de alga, y temperatura) en la demografía de *Alona guttata* (Sars, 1862) para obtener sus mayores tasas de crecimiento. **Métodos.** Se realizaron análisis de tablas de vida con el quidórido *A. guttata* de la manera siguiente: cinco hembras por cohorte (seis réplicas) fueron alimentadas con *Chlorella vulgaris* (Beijerinck, 1890) o *Nannochloropsis oculata* (Hibberd, 1981), a una concentración de 0.5×10^6 o 2×10^6 células/mL, con una temperatura de 20°C o 25°C, y un fotoperiodo de 16:8 h (luz:oscuridad). Al medio de cultivo se adicionó un sustrato artificial. Posteriormente, la fertilidad y supervivencia diarias fueron evaluadas para estimar los parámetros poblacionales: promedio de vida (ALS), expectativa de vida al nacimiento (LEB), tiempo generacional (GT), tasa reproductiva bruta (GRR), longevidad, tasa reproductiva neta (NRR), y la tasa intrínseca de crecimiento poblacional (r). **Resultados.** Se observaron efectos significativos debidos a los tres factores evaluados y sus interacciones. El aumento de la concentración de las algas *C. vulgaris* o *N. oculata* incrementó la fertilidad y supervivencia. La menor temperatura extendió la ALS y la LEB cuando los organismos fueron alimentados con la concentración más alta de algas. Las r más altas se observaron cuando *Alona* fue alimentada con *N. oculata* a 2×10^6 células/mL. **Conclusiones.** Las mejores condiciones de cultivo, en términos de la tasa de crecimiento poblacional de *A. guttata*, fueron provistas por *N. oculata* a 2×10^6 cells/mL, a 25°C, con la adición del sustrato artificial.

Palabras clave: análisis de tabla de vida, Anomopoda, fertilidad, parámetros demográficos, supervivencia

INTRODUCTION

Chydoridae is the most diverse family within cladocerans, but information regarding their culture and biology is still scarce in comparison to other families like Daphniidae and Moinidae. In natural environments, fish larvae can feed on little size species like chydorids, which are preferably consumed over other large cladocerans, likely because of their ease to capture (Nunn *et al.*, 2012). Chydorids, which inhabit littoral zones of water bodies, are generally associated to aquatic plants, periphyton, or sediment (Dole-Olivier *et al.*, 2000; Masclaux *et al.*, 2012). Thus, different media have been developed to culture chydorids in laboratory conditions, feeding these organisms on algae, bacteria, detritus, yeast, or combinations of these sources of organic matter (Martínez-Jerónimo & Gomez-Díaz, 2011).

Castilho *et al.* (2015) stated that the study of the life cycle of cladocerans offers a better understanding of their biology, and at higher hierarchy level provide information about population dynamics, their interactions with the surroundings, and their role within food webs and secondary production.

Alona guttata (Sars, 1862) is a cosmopolitan species, with records in several places around the world (Sinev & Siva-Briano, 2012; Sousa *et al.*, 2014). Cortez-Silva *et al.* (2022) described the life history of *A. guttata* fed on *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J.Kristiansen & O.M.Skulberg 1987, and the strategies that this species might follow to rapidly colonize temporal and transitory ponds. Alvarado-Suárez (2017) implemented this chydorid to nourish the goodeid fish *Poeciliopsis infans* (Woolman, 1894) and concluded that *A. guttata* is of high nutritional value but their usage is limited due to poor biomass production. Therefore, this study was aimed to assess the effect of the food source, algal concentration, and temperature on the demography of *A. guttata* through life table analysis and find the best culture conditions in terms of the population growth rates.

MATERIAL AND METHODS

Maintenance of chydorids. The strain of *A. guttata* has been maintained in the Laboratory of Aquatic Toxicology of the Universidad Autónoma de Aguascalientes (UAA) and was cultured with the following conditions: moderately hard reconstituted water (MHRW) (USEPA, 2002), photoperiod 16h light and 8h dark, fed once a day *ad libitum* with either the green algae *Chlorella vulgaris* (Beijerinck, 1890) or *Nannochloropsis oculata* (Hibberd, 1981), and temperature at either 20°C or 25°C ± 2°C. Freshwater media was supplemented with 660 mg/L of artificial substrate, which consisted of 70% silica sand, 25% kaolin, and 5% dried and ground cattle manure. The complete mixture of the substrate was sterilized by autoclave at 15 psi for 20 min (Martínez-Jerónimo & Gómez-Díaz, 2011).

Algal biomass as food source for chydorids. *Chlorella vulgaris* and *N. oculata* were grown in Bold's Basal medium (Nichols, 1973) under aseptic conditions at 25°C. Continuous illumination was provided by day-light fluorescent lamps (approximately 5000 lux). Algal biomass was collected during the exponential phase of population growth and separated from the culture medium by centrifugation at 5000 rpm for 5 min. Then, culture media for chydorids were supplemented with either *C. vulgaris* or *N. oculata* at low (0.5×10^6 cells/mL) and high densities (2×10^6 cells/mL).

Effect of diet and temperature on *Alona guttata*. Cohorts of five neonates (less than 24-h old) of *A. guttata* were placed separately in a 24-well polystyrene microplate; then, every well was supplemented with the corresponding algal suspension in MHRW and adjusted to a final volume of 2 mL. Temperature was controlled in bioclimatic chambers at either 20°C or 25°C ± 2°C with a photoperiod of 16 h light and 8 h dark. To avoid desiccation, 200 µL of distilled water were added every other day to each well. Complete renewal of the culture media was performed once a week. Fertility and survival data were daily recorded until all individuals in the cohort had died. The neonates and dead organisms were removed and counted daily. All treatments consisted of six replicas (n = 6).

Life table analysis. Data of survival (l_x) and fertility (m_x) were used to determine: average lifespan (ALS) (days), maximum longevity (days), life expectancy at birth (LEB) (days), generation time (GT) (days), gross reproductive rate (GRR) (neonates/female), net reproductive rate (NRR) (neonates/female), and intrinsic rate of population increase (r) (1/days) (Pianka, 1978; Krebs, 1985):

$$\text{Average lifespan ALS} = \sum l_x$$

$$\text{Life expectancy at birth LEB} = \frac{T_x}{l_x}$$

$$\text{Gross reproductive rate GRR} = \sum_0^{\infty} m_x$$

$$\text{Net reproduction rate NRR} = \sum_0^{\infty} l_x m_x$$

$$\text{Generation time GT} = \frac{\sum l_x m_x x}{\text{NRR}}$$

The intrinsic rate of population increase (IRPI) was computed through iteration with the Euler-Lotka equation: $\sum_{x=0}^{\infty} e^{-rx} l_x m_x = 1$

Statistical Analysis. Results from the life table experiments were analyzed through three-way analysis of variance (ANOVA). Significant differences were established through Bonferroni's multiple comparison test. Statistical analysis were performed in R-Studio v.1.0.143 and the packages *agricolae* v.1.2-4 (De Mendiburu, 2016) and *ggplot2* v.3.2.1 (Wickham, 2016).

RESULTS

Figure 1 shows the survival curves for *A. guttata* raised at 20°C or 25°C and fed on 0.5×10^6 or 2×10^6 cells/mL of either *C. vulgaris* or *N. oculata*. The longer survival rates were recorded when organisms were reared at 20°C in medium supplemented with *N. oculata*. Chydorids fed on *N. oculata* (2×10^6 cells/mL) and grown at 20°C delayed until the day 40 of the test to exhibit the first females to die, while at 25°C dead organisms appeared since the day 30. Temperature alone seemed to have no significant effect on the survival of *A. guttata*, but it was influenced by the interaction of temperature × algal concentration and temperature × algal species.

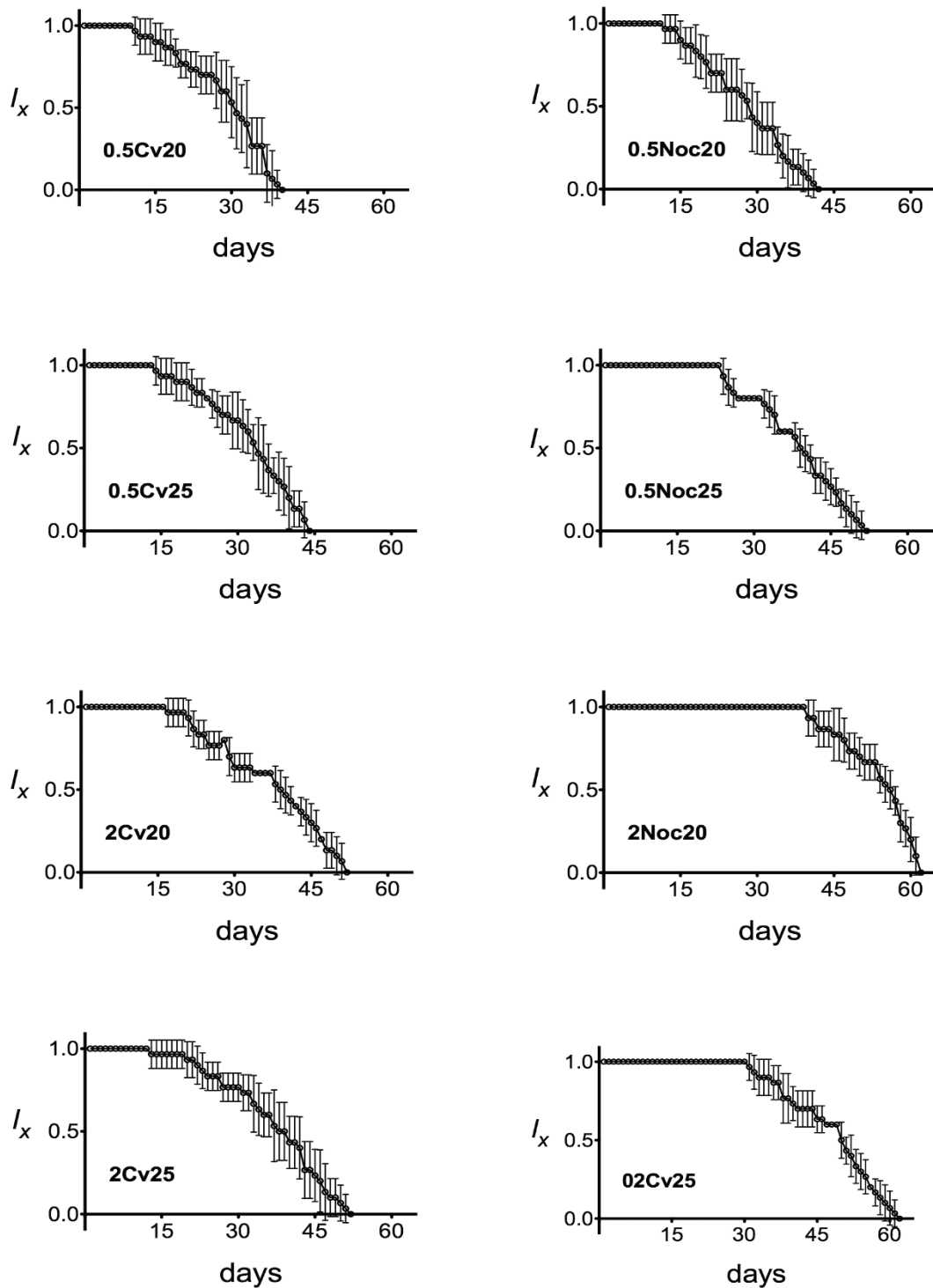


Figure 1. Survival (I_x) of *Alona guttata* (Sars, 1862) fed on *Chlorella vulgaris* (Beijerinck, 1890) and *Nannochloropsis oculata* (Hibberd, 1981).

Error bars represent the confidence interval ($P < 0.05$). All experiments were carried out with six replicates ($n = 6$). 0.5 = 0.5×10^6 cells/mL; 2 = 2×10^6 cells/mL; Cv = *C. vulgaris*; Noc = *N. oculata*; 20 and 25 = temperature in Celsius.

Figure 2 shows the daily fertility of the eight treatments. *Alona guttata* produced offspring during almost their entire life cycle, although the number of neonates per reproductive episode was in general low. Maximum values of longevity were recorded for every treatment, with the highest values (60.33 ± 0.8164 d) when organisms were fed on *N. oculata* at 2×10^6 cells/mL (Fig. 2). The highest longevity was recorded

for the group fed on *C. vulgaris* (49.17 ± 1.8348 d). In general, the concentration of 0.5×10^6 cells/mL proved to be insufficient to promote longer longevity in *Alona*. These results were influenced mainly by the algal species and the algal concentration but were not significantly affected by temperature (Table 1).

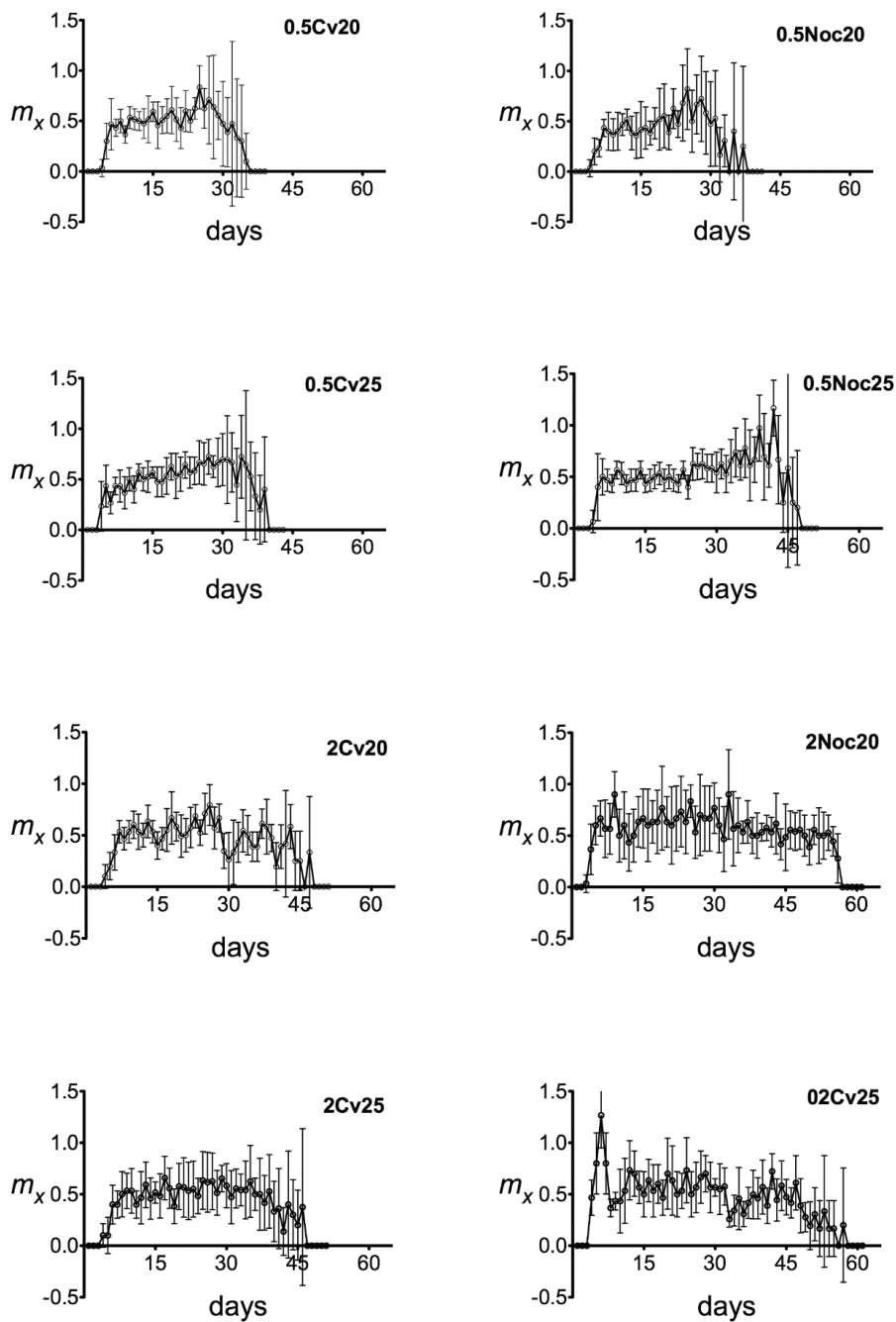


Figure 2. Fertility (m_x) of *Alona guttata* (Sars, 1862) fed on *Chlorella vulgaris* (Beijerinck, 1890) and *Nannochloropsis oculata* (Hibberd, 1981).

Error bars represent the confidence interval ($P < 0.05$). All experiments were carried out with six replicates ($n = 6$). 0.5 = 0.5×10^6 cells/mL; 2 = 2×10^6 cells/mL; Cv = *C. vulgaris*; Noc = *N. oculata*; 20 and 25 = temperature in Celsius.

Table 1. Analysis of variance performed on the demographic responses of *Alona guttata* fed on *Chlorella vulgaris* or *Nannochloropsis oculata*. ALS, average lifespan (days); longevity, maximum longevity recorded by replicate (days); LEB, life expectancy at birth (days); GT, generation time (days); GRR, gross reproductive rate (neonates/female); NRR, net reproductive rate (neonates/female); IRPI, intrinsic rate of population increase, 1/days.

	Factor or interaction	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
ALS	Algal species	1	585.20	585.20	86.207	< 0.001	***
	Algae concentration	1	2,192.40	2,192.40	322.966	< 0.001	***
	temperature	1	7.70	7.70	1.131	0.294	
	Algal species × algae concentration	1	588.00	588.00	86.619	< 0.001	***
	Algal species × temperature	1	9.40	9.40	1.379	0.247	
	Algae concentration × temperature	1	162.80	162.80	23.983	< 0.001	***
	Algal species × algae concentration × temperature	1	40.30	40.30	5.942	0.019	*
	Residuals	40	271.50	6.80			
Longevity	Algal species	1	574.10	574.10	123.903	< 0.001	***
	Algae concentration	1	2,352.00	2,352.00	507.626	< 0.001	***
	temperature	1	36.80	36.80	7.932	0.007	**
	Algal species × algae concentration	1	225.30	225.30	48.633	< 0.001	***
	Algal species × temperature	1	0.70	0.70	0.162	0.689	
	Algae concentration × temperature	1	161.30	161.30	34.82	< 0.001	***
	Algal species × algae concentration × temperature	1	0.30	0.30	0.072	0.79	
	Residuals	40	185.30	4.60			
LEB	Algal species	1	585.20	585.20	86.207	< 0.001	***
	Algae concentration	1	2,192.40	2,192.40	322.966	< 0.001	***
	temperature	1	7.70	7.70	1.131	0.294	
	Algal species × algae concentration	1	588.00	588.00	86.619	< 0.001	***
	Algal species × temperature	1	9.40	9.40	1.379	0.247	
	Algae concentration × temperature	1	162.80	162.80	23.983	< 0.001	***
	Algal species × algae concentration × temperature	1	40.30	40.30	5.942	0.019	*
	Residuals	40	271.50	6.80			
GT	Algal species	1	38.00	38.00	23.624	< 0.001	***
	Algae concentration	1	287.00	287.00	178.412	< 0.001	***
	temperature	1	2.13	2.13	1.327	0.256	
	Algal species × algae concentration	1	22.12	22.12	13.754	< 0.001	***
	Algal species × temperature	1	8.80	8.80	5.469	0.024	*
	Algae concentration × temperature	1	44.93	44.93	27.929	< 0.001	***
	Algal species × algae concentration × temperature	1	15.82	15.82	9.832	0.003	**
	Residuals	40	64.34	1.61			
GRR	Algal species	1	0.50	0.50	0.12	0.731	
	Algae concentration	1	148.50	148.50	35.662	< 0.001	***
	temperature	1	318.10	318.10	76.38	< 0.001	***
	Algal species × algae concentration	1	1.90	1.90	0.467	0.498	
	Algal species × temperature	1	161.10	161.10	38.68	< 0.001	***
	Algae concentration × temperature	1	11.30	11.30	2.724	0.107	
	Algal species × algae concentration × temperature	1	104.30	104.30	25.052	< 0.001	***
	Residuals	40	166.60	4.20			

	Factor or interaction	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
NRR	Algal species	1	254.80	254.80	180.248	< 0.001	***
	Algae concentration	1	875.50	875.50	619.253	< 0.001	***
	temperature	1	1.30	1.30	0.896	0.349	
	Algal species × algae concentration	1	404.80	404.80	286.343	< 0.001	***
	Algal species × temperature	1	9.90	9.90	7.003	0.012	*
	Algae concentration × temperature	1	106.20	106.20	75.12	< 0.001	***
	Algal species × algae concentration × temperature	1	33.70	33.70	23.813	< 0.001	***
	Residuals	40	56.60	1.40			
IRPI	Algal species	1	0.00696	0.00696	48.194	< 0.001	***
	Algae concentration	1	0.01844	0.01844	127.632	< 0.001	***
	temperature	1	0.00109	0.00109	7.541	0.009	**
	Algal species × algae concentration	1	0.02120	0.02120	146.711	< 0.001	***
	Algal species × temperature	1	0.00108	0.00108	7.452	0.009	**
	Algae concentration × temperature	1	0.00009	0.00009	0.654	0.424	
	Algal species × algae concentration × temperature	1	0.00039	0.00039	2.71	0.108	
	Residuals	40	0.00578	0.00014			

From the life table analysis, *Alona guttata* showed the longest ALS values when feeding on algal densities of 2×10^6 cells/mL (Fig. 3). Significant interactions were recorded for algal species × algal concentration ($P < 0.01$), algal concentration × temperature ($P < 0.01$), algae species × algae concentration × temperature ($P < 0.05$) (Table 1). The ALS presented values from 26.97 (SD = 3.2752) to 53 d (SD = 1.8199), with *N. oculata* as the best food source for this chydorid when it was supplemented at 2×10^6 cells/mL.

The life expectancy at birth (LEB) was mainly affected by the algal species and algae concentration; thus, obtaining the highest LEB value (52.50 d) with *N. oculata* (2×10^6 cells/mL) at 20°C (Fig. 3). Significant interactions were registered for algae concentration × algal species, algae concentration × temperature, and for the three factors tested (Table 1).

The highest GT (27.28 ± 0.8018 days) was recorded when chydorids fed on *N. oculata* (2×10^6 cells/mL) and reared at 20°C (Fig. 3). The lowest GTs were observed with the organisms fed on either *C. vulgaris* or *N. oculata* at 0.5×10^6 cells/mL. The two temperatures tested caused no significant differences in the GT of *A. guttata*, although their interactions significantly affected the GT (Table 1).

GRR was influenced by the algal concentration of both algal species (Table 1); then, organisms fed on 0.5×10^6 cells/mL produced fewer neonates than the organisms fed on 2×10^6 cells/mL. Thereafter, *N. oculata* at the highest algal density promoted *A. guttata* to have more neonates during their whole life cycle (Fig. 3).

The NRR registered the highest value with the females fed on *N. oculata* at 2×10^6 cells/mL (28.86 ± 0.8547 neonates/female), while those organisms fed on *C. vulgaris* produced fewer neonates, even when *C. vulgaris* was supplemented at 2×10^6 cells/mL (Fig. 3).

Finally, the treatment that promoted the higher rates of population increase was *N. oculata* at 2×10^6 cells/mL and temperature at 25°C ($r = 0.2656 \pm 0.0163$). The fed on *C. vulgaris* did not reach the same population growth rates in comparison to the groups fed on *N. oculata*.

Alona guttata fed on *C. vulgaris* exhibited similar growth rates than those females fed on *N. oculata* at 0.5×10^6 cells/mL (Fig. 3).

DISCUSSION

Although there are several reports on chydorid species, information on their life cycle or reproductive biology is still scarce. Therefore, the main contributions of this study are: a) the insights on *A. guttata* asexual reproduction since neither males nor ephippia were detected while carrying out the different experiments during this research; and b) the culture conditions that promoted the highest rates of population increase to obtain higher number of individuals for further usage, as food source for early life stages of fish (Alvarado-Suárez, 2017) or as test organisms in environmental toxicology (Garza-León *et al.*, 2017; Osorio-Treviño *et al.*, 2019).

The maximum survival of *A. guttata* was comprised within values reported for other chydorids (Table 2). The longest survival within the family Chydoridae were reported for organisms grown at 5°C, reaching values of about 100 d. In the subfamily Aloninae survival has registered values of up to 90 d. For instance, Cortez-Silva *et al.* (2022) reported that longevity of *A. guttata* reached 37 d as maximum (30.9 d average) when chydorids were fed on *R. subcapitata*, but we found that this species can survive longer when feeding on either *C. vulgaris* or *N. oculata*. It is worth pointing out that food source and temperature influenced longevity of organisms and several publications reported no more than one food source or temperature. Although several results showed long survival of chydorids, some others might be explored to improve the survival of organisms.

The NRR in chydorids can take values from 6 to 135 neonates per female, with the highest reproduction rates in the genus *Eurycerus* (Table 2). In *A. guttata* the NRR was influenced by the algae concentration and the algal species, reaching the highest values at 2×10^6 cells/mL with *N. oculata* as food source. On this regard, Muro-Cruz *et al.*

(2002) found no significant differences in the NRR of *Coronatella rectangularis* (Sars, 1861) (formerly *Alona rectangularis*) when feeding on *C. vulgaris* at 0.5 and 2×10^6 cells/mL (NRR = 14.72 ± 0.62 and 13.22 ± 0.77 neonates/female, respectively); however, *A. guttata* showed sig-

nificant differences when fed on increasing algal concentrations, reaching higher reproductive rates, GRR and NRR, when organisms were fed on *C. vulgaris* or *N. oculata* at 2×10^6 cells/mL, which improved the reproductive performance of *A. guttata*.

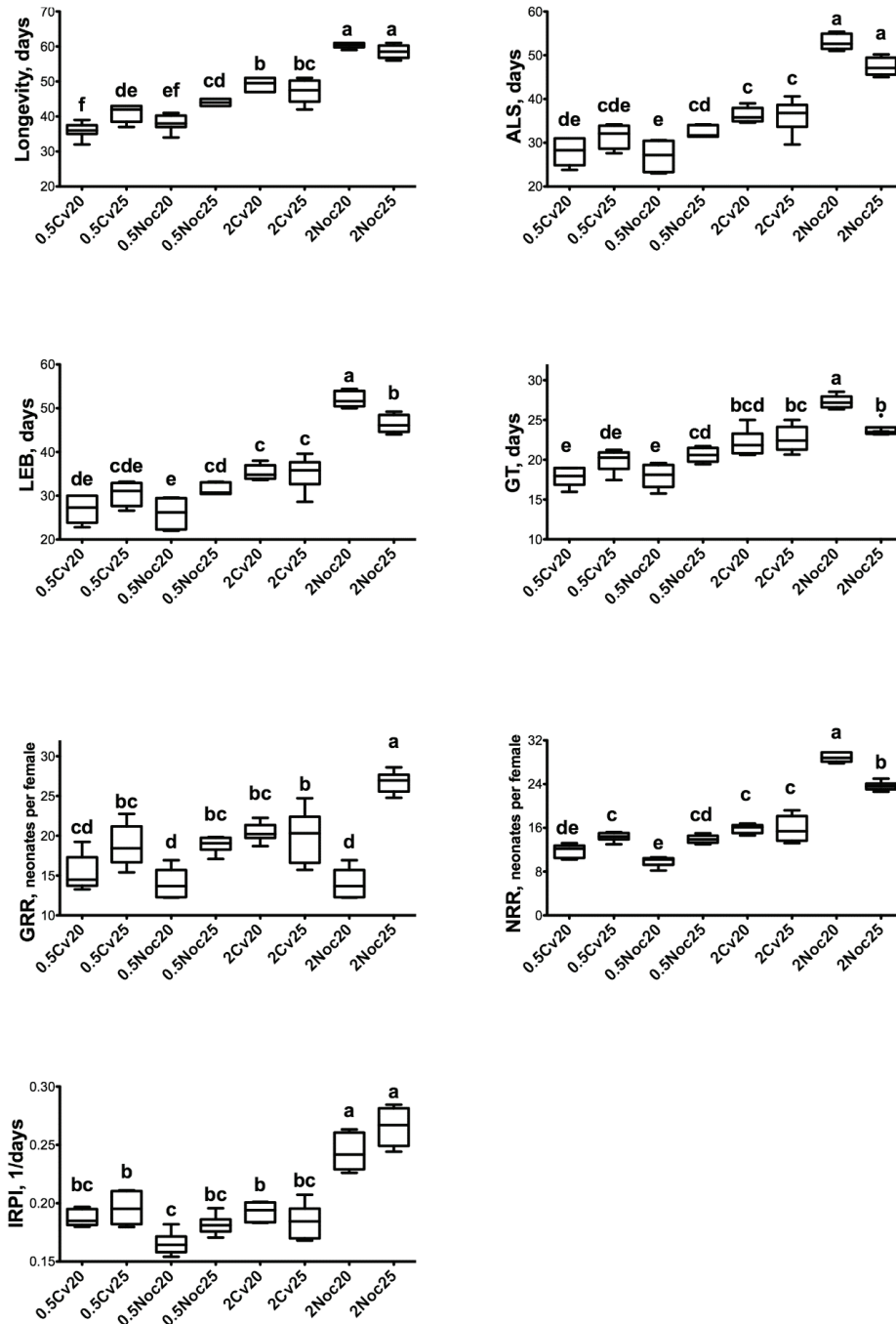


Figure 3. Demographic responses of *Alona guttata* fed on *Chlorella vulgaris* (Beijerinck, 1890) and *Nannochloropsis oculata* (Hibberd, 1981). ALS = average life span, GT = generation time, LEB = life expectancy at birth, GRR = gross reproductive rate, NRR = net reproductive rate, and IRPI = rate of population increase. All experiments were carried out with six replicates (n = 6). 0.5 = 0.5×10^6 cells/mL; 2 = 2×10^6 cells/mL; Cv = *C. vulgaris*; Noc = *N. oculata*; 20 and 25 = temperature in Celsius. Significant differences were established by factorial ANOVA and multiple comparison test of Bonferroni ($P < 0.05$)

Table 2. Comparison of the life cycle and demographic responses of chydorids from this study and reported in the literature. GT, generation time (days); GRR, gross reproductive rate (neonates/female); NRR, net reproductive rate (neonates/female); IRPI, intrinsic rate of population increase; T, temperature

Species	T (°C)	Survival (d)	GT	GRR	NRR	IRPI	Reference
<i>Acroperus harpae</i> (Baird, 1834)	5 – 25	9 – 119	5.3 – 70.4				Bottrell, 1975
<i>Alona affinis</i> (Leydig, 1860)	5 – 20	37 – 144	16.9 – 72.9				Bottrell, 1975
<i>A. guttata</i> (Sars, 1862)	22	37					Cortez-Silva <i>et al.</i> , 2002
	20 – 25						This study
<i>A. iheringula</i> (Sinev & Kotov, 2004)	25	54	5.0				Silva <i>et al.</i> , 2014
<i>Alonella excisa</i> (Fischer, 1854)		73					Sharma & Sharma, 1998
<i>Chydorus pubescens</i> (Sars, 1901)	23.6	31	4.3				Santos-Wisniewski <i>et al.</i> , 2006
	5 – 20	23 – 96	8.9 – 38.5				Bottrell, 1975
<i>C. sphaericus</i> (Müller, 1776)	15 – 25	26.92 – 30.82	10.3 – 24.9	19.04 – 34.96	0.143 – 0.268		Keen, 1967 (cited by Hann 1985)
	25	28.4	10.2 – 10.6	18.0 – 18.8	0.25 – 0.26		Muro-Cruz <i>et al.</i> , 2002
<i>Coronatella rectangulara</i> (Sars, 1862)	23.6	30 – 80	4.2				Viti <i>et al.</i> , 2013
<i>Disparalona rostrata</i> (Koch, 1841)	10 – 19	24	18.8 – 35.5				Robertson, 1988
<i>Euryalona orientalis</i> (Daday, 1898)	28–30	42 – 162	19.1 – 100.5				Venkataraman, 1990
<i>Euryercus lamellatus</i> (Müller, 1776)	5 – 20	16 – 19	16.9 – 28.3	10.45 – 13.25	0.083 – 0.153		Bottrell, 1975
<i>E. longirostris</i> (Hann, 1982)	16 – 24	16 – 17	16.5 – 32.5	8.53 – 10.12	0.071 – 0.130		Hann, 1985
<i>E. vernalis</i> (Hann, 1982)	10 – 25	35.50 – 79.7	15.2 – 53.2	69 – 135	0.08 – 0.310		Lemke & Benke, 2004
<i>Graptoleberis testudinaria</i> (Fischer, 1851)	5 – 20	23 – 95	9.5 – 44.6				Bottrell, 1975
<i>Karualona muelleri</i> (Richard, 1897)	23 – 26	17			0.180		Panarelli <i>et al.</i> , 2019
<i>Leydigia acanthocercoides</i> (Fischer, 1854)	29	23	4.7				Murugan & Job, 1982
<i>L. ciliata</i> (Gauthier, 1939)	28–30	46					Venkataraman, 1990
<i>L. leydigi</i> (Schödler, 1863)	5 – 19	21 – 120	10.1 – 63.9				Robertson, 1988
<i>L. lousi mexicana</i> (Kotov, Elias-Gutiérrez & Nieto, 2003)	20 – 25	58 – 97	18.2 – 36.8	17.34 – 24.86	0.120 – 0.190		Martínez-Jerónimo & Gómez-Díaz, 2011
<i>Oxyurella longicaudis</i> (Birge, 1910)	23	47	7.50				Castilho <i>et al.</i> , 2015
<i>Pleuroxus aduncus</i> (Jurine, 1820)	25	16 – 44	7.4 – 14.4	1.04 – 13.8	6.47 – 0.53	-0.091 – 0.149	Nandini & Sarma, 2000
<i>P. denticulatus</i> (Birge, 1879)	15 – 25	31 – 132	8.4 – 25	4.92 – 10.96	0.096 – 0.174		Keen, 1967 (cited by Hann 1985)
<i>P. uncinatus</i> (Baird, 1850)	5 – 20	13.6 – 78.3					Bottrell, 1975

The GT in chydorids varies significantly as a function of food source, food density, and temperature, taking values from some days (about 10 d) up to 100 d (Table 2). The longest GT were reported for organisms within the subfamily Chydorinae, like *Acroperus harpae* (Baird, 1834) and *Eurycerus lamellatus* (Müller, 1776) when they were grown at 5 °C, but increasing temperature to 25°C promoted significantly lower values of 5.30 d and 19.08 d, respectively. In the subfamily Aloninae, the longest GT was reported for *Alona affinis* (Leydig, 1860) (72.88 d at 5°C) while *C. rectangula* presented the shorter GT (4.16 d at 23.6°C) (Table 2). Thus, the GT of *A. guttata* (17.82 ± 1.15 to 27.28 ± 0.80 d) is similar to the values reported for other chydorid species. Bottrell (1975) stated that the interaction of abundant food supply and increasing temperature promote shorter times for every developmental stage in chydorids, thus, the GT can be shorten as it was observed within the group of *A. guttata* fed on *N. oculata* (2×10^6 cells/mL). On the other side, Muro-Cruz *et al.* (2002) found no significant differences for the GT of *C. rectangula* fed on *C. vulgaris* at either 0.5×10^6 or 2×10^6 cells/mL.

For chydorids, the intrinsic rate of population increase (r) is generally accepted to take low values, which are in most cases below or near 0.2/d (Martínez-Jerónimo & Gómez-Díaz, 2011; Nandini *et al.*, 2007), with the exception of some species of the genus *Eurycerus*, which can reach high values for chydorids of up to 0.310/d (Table 2). Furthermore, the intrinsic rates of population increase are affected by temperature, for instance, Hann (1985) found that increasing temperatures promoted higher reproductive rates in chydorids by decreasing egg maturation times; therefore, in four species the highest “ r ” values were recorded at 24 – 25°C. Despite *A. guttata* presented high growth rates in comparison to *Pleuroxus aduncus* (Jurine, 1820) 0.15/d (Nandini & Sarma, 2000), or very similar to those of *C. rectangula* or *Karualona muelleri* (Richard, 1897) (Muro-Cruz *et al.*, 2002; Panarelli *et al.*, 2019), such values are below the intrinsic growth rates that other cladocerans like *Moina* sp. can reach (Sipaúba-Tavares & Bachion, 2002; Deng & Xie, 2003; Rodríguez-Estrada *et al.*, 2003).

In relation to algal density, some cladoceran species are adapted to either low or high densities; for instance, *C. rectangula* and *P. aduncus* showed lower growth rates at increasing algal densities (Nandini & Sarma, 2000; Muro-Cruz *et al.* 2002). Nevertheless, these authors tested a single algal species. In our study, *A. guttata* exhibited higher growth rates with increasing algal density, with *N. oculata* as a better food source over *C. vulgaris*.

The algal species has also significant effects on the reproduction and survival of *A. guttata*. In this study we observed that either *C. vulgaris* or *N. oculata* were good food source, which promoted a higher performance than the alga *R. subcapitata*.

In the literature, the genera *Chlorella* and *Nannochloropsis* have been described to synthesize high amounts of polyunsaturated fatty acids (PUFA) in comparison to other genera like *Raphidocelis* (Patil *et al.*, 2007); therefore, feeding on these algae could serve as a better food source that provides the essential nutrients to enhance reproduction and survival (Schlotz *et al.*, 2012).

Up to date, three publications have reported the survival and fertility of *A. guttata*. On the one side, Garza-León *et al.* (2017) and Osorio-Treviño *et al.* (2019) carried out partial life table analysis with similar algal densities (*N. oculata* at 2×10^6 cells/mL) and temperature (25°C) than the present study; on the other side, Cortez-Silva *et al.* (2022)

studied the life cycle but used *R. subcapitata* as food source and temperature within the interval here tested. In these studies, neither of them employed any substrate in the culture media, and despite *A. guttata* survived and produced offspring in those conditions, we found out that the inclusion of substrate improved the performance of these chydorids, increasing their rate of population growth and survival. It has been demonstrated that not all chydorids grow better on a diet composed solely of microalgae but rather on one of detritus or bacteria (Smirnov, 1962; Vijverberg & Boersma, 1997), although a mixed diet could be more important for chydorids nutrition (Lemke *et al.*, 2007).

In conclusion, *A. guttata* can feed on either *C. vulgaris* or *N. oculata*, increasing their longevity and fertility when algal density is high (2×10^6 cells/mL). Temperature had significant effects on the life history of *A. guttata*, but it was the interaction with the algal density and algal species which produced more significant effects on the performance of *A. guttata*. The continuous production of offspring and long survival in *A. guttata* allowed intrinsic growth rates that are among the highest within the family Chydoridae, which might suggest this species to be cultured for further purposes like aquaculture (since some *Alona* species form part of fish diets) or ecotoxicological studies, which have already been reported in the literature.

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