

***Ceriodaphnia dubia* (Richard 1894) and *Daphnia pulicaria* (Forbes, 1893), fed with *Sphaerocystis* sp. and *Chlorolobion* sp. microalgae's for laboratory production.**

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ABSTRACT

One of the most used microorganisms in this industry are cladocerans, which can be produced in easy culture systems. *C. dubia* and *D. pulicaria*, which are microalgae, filter feeders organisms like *Sphaerocystis* sp. and *Chlorolobion* sp. These cladocerans were culture during 91 days in 20 L beakers, fed separately with two microalgae and one mixed diet. The three diets were maintain at 500×10^3 cells mL^{-1} . The population density was 1 to 227 org mL^{-1} . The mixed diet with those two microalgae obtained the best density results with *C. dubia* and with *D. pulicaria* with *Sphaerocystis* sp. diet. Each female produced 24 to 3,789 new organisms and have a reproduction rate of 0.54 to 0.84; generation time have 5.96 to 9.80 day range. These species show adequate size to consider as potential live food to aquarist industry because they are able to adapt to laboratory production conditions, where abiotic factors can be controlled and different microalgae diets can applied to improve better potential productions with both cladocerans populations.

Key words: Cladocerans, life tables, tendency curves, microalgae.

RESUMEN

El alimento vivo en acuicultura juega un papel importante dentro del desarrollo de los organismos acuáticos. Dentro del grupo que más se utiliza están los cladóceros, los cuales pueden ser de fácil cultivo. Dentro de este grupo están las especies *C. dubia* y *D. pulicaria*, las cuales son filtradoras de microalgas unicelulares como el

Sphaerocystis sp. y el *Chlorolobion* sp. Los cultivos de estos organismos zooplanctónicos se llevaron a cabo durante 91 días en recipientes de 20 L, alimentados con estas dos microalgas por separado y una dieta combinada de ambas microalgas (500×10^3 cél mL^{-1}). Se obtuvieron densidades desde 1 mL^{-1} hasta 227 mL^{-1} . Siendo mejor dieta la combinada con *C. dubia* y en *D. pulicaria* con *Sphaerocystis* sp. Los organismos producidos por hembra estuvieron en el rango de 24-3,789 por hembra, con tasas de reproducción (r) de 0.54-0.84. El tiempo generacional fluctuó entre 5.96 a 9.80 días. Estas dos especies de cladóceros presentan características de tamaño adecuado para ser considerados como potenciales alimentos para acuicultura ya que se adaptan a condiciones de producción en laboratorio, donde los factores ambientales pueden ser controlados y se pueden suministrar diversas dietas para mejorar parámetros reproductivos de ambas poblaciones.

Palabras clave: Cladóceros, tablas de vida, curvas de crecimiento, microalgas.

INTRODUCTION

Live food production plays an important role in development in some aquatic organisms, principally larviculture, either as total or complement diet. Inert diet like pellets or flakes are not totally consumed by aquatic organisms or it does not cover the nutritional requirements for aquaculture or aquariophylla organisms. Live food production is not an easily practice. It has different characteristics that make it attractive to

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fish and crustaceans like: better distribution in column water, does not damage aquatic environment, their nutritional composition can be modified, it has different life stages with different sizes that makes it more useful, a better digestibility or does not dissolved or break up in water medium like pellets or flakes. These characteristics avoid decomposition of culture medium (Ocampo et al. 2012).

The supply of live food gives better results in larviculture of fish and crustaceans, which are the most difficult stage before moving on to inert type food supply, resulting in higher levels of survival, development and sexual maturity in aquatic organisms in culture (Ocampo et al. 2010).

It is common that zoo planktonic species like ciliates, rotifers, copepods, ostracods, cladocerans and *Artemia* are use as live food in larvicultura and aquariophylia for their culture facility system and their capacity to change their nutritional values (Castro et al. 2013). In cladocerans group we can find two freshwater flies that can be important due to their easy production like *Ceriodaphnia dubia* and *Daphnia pulex*.

D. pulex founded principally at the bottom of water bodies where they live and are more abundant at springtime^[3, 4]. (Brandlova et al. 1972; Stich and Maier 2007). The juvenile and adult stage are distribute according to availability and quality of food, and by the presence of their predators (Reichwaldt and Abrusán 2007; Reichwaldt 2008). The juvenile organisms founded principally in the epilimnion where water is warmer, meanwhile bigger adult stage and depredators can find them easily, were located at hipolimnion principally (Reichwaldt 2008). Adult organisms showed more vertical migration and they were located at surface water at night (Cerny y Bytel 1991; Leibold 1991). *D. pulex* can reproduced in a parthenogenetic

way, principally and only when environmental conditions are adverse, it presents sexual reproduction. These sexual adults produced latency eggs or ephippia, which can resist dry and freeze seasons until environmental conditions are favorable (Chen y Felt 1996; Brewer 1998).

C. dubia was located at shore of freshwater bodies. They are organisms <1 mm size. The male stage are smaller than female stage. The second antennae is larger than body length, which serve to propel swimming. This specie used principally to make toxicity tests with wastewater.

These two cladocerans species have characteristics of suitable size considered as potential live food for aquaculture or aquariophylia, because they can adapt to laboratory production conditions, where environmental factors can controlled and can supplied different diets to improve reproductive parameters. For this reason, the mean goal of this study was to use *Sphaerocystis* sp. and *Chlorolobion* sp., two green microalgae that have not used as a common food in this type of cladocerans, to assess their population dynamics and obtain information for maintenance in laboratory and their possible massive production.

MATERIAL AND METHODS

Samples of *C. dubia* and *D. pulex*

Water samples were collect from Xochimilco water channels in Mexico City (Fig.1), with 1.0, 0.5 and 0.25 nylon mesh, to obtain different cladocerans stages. For taxonomic identification, the Hanney's keys Haney et al. (2013) (<http://cfb.unh.edu/cfbkey/html/index.html>)

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were used. The most abundant cladocerans were *C. dubia* and *D. pulicaria* with which we proceeded to make experiments.

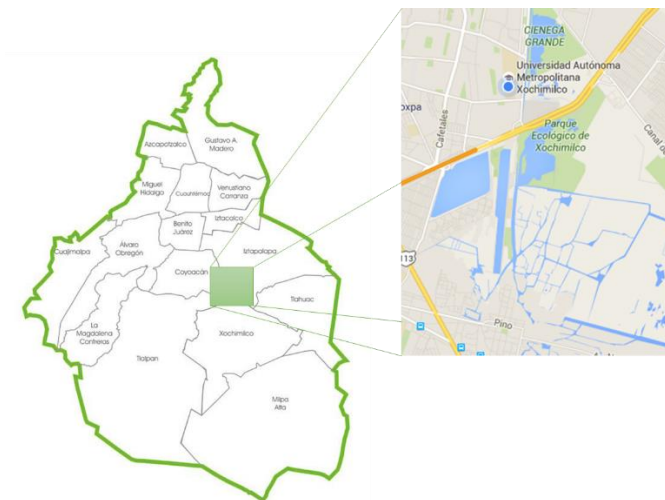


Fig.1: Geographical localization of Xochimilco water channels, Mexico City.

Experimental design

According environment conditions in their own habitat, *C. dubia* was maintain at 19°C temperature and *D. pulicaria* at 24°C. For culture of both cladocerans, 20 L beakers were use with a pH 7-8, with continuous light and aeration during all experiment time. Because of their different size, *C. dubia* initiated with 60 organisms at juvenile stages, meanwhile *D. pulicaria* started with 30 juvenile stages organisms per beaker. The experiment was made by triplicate to obtain mean values (\pm S.D.) of produced organisms (density). Experiment lasted 91 days for both species (Fig.2).

Food supply

For both experiments it was used two green microalgae *Sphaerocystis* sp. and *Chlorolobion* sp. at 500×10^3 cells mL⁻¹ density,

supplied separately and third one as a combined diet with those two microalgae at same concentration (1:1). Every third day, 100 mL of these diets were supplied to culture systems. Each week, microalgae culture system were 50% harvested, filled again with tap water and fertilized with 20 mL of Triple 17 (50 g 500 mL⁻¹ of tap water) and 5 mL of Urea (1 Kg 4L⁻¹ of tap water) to maintained microalgae cells concentration per milliliter. Microalgae density was check by counting number of cells per milliliter with Neubauer chamber, organisms fed previously.

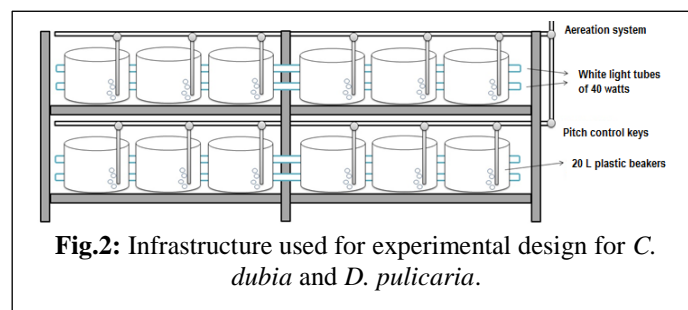


Fig.2: Infrastructure used for experimental design for *C. dubia* and *D. pulicaria*.

Sampling

Each week 100 mL from each 20 L beaker was take to count the total number of organisms and extrapolated this number to 1 liter.

Processing data

With obtained values, a database was made in Excel 2010 program from each cladocerans specie. A descriptive analysis was made per each week. A tendency grow curves were made. With mean values, it was proceeded to make a Life Table from each cladocerans specie. Used formulas were:

Reproduction rate (Ro):

$$Ro = \sum_l x m_x$$

Where:

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l_x = Survival proportion in each life phase.
 m_x = Organisms produced in each phase/Number of organisms observed in each phase.

Generational Time of Cohort (GTC):

$$GTC = \sum l_x m_x / R_0$$

Where:

x = phase.

$\sum l_x m_x$ = Summary of produced organisms for each single organism at each phase.

R_0 = reproduction rate.

Instantaneous grow rate (r):

$$r = \log_e R_0 / T_c$$

Where:

$\log_e R_0$ = Logarithm e base of reproduction rate.

GTC= Generation time of cohort.

Survival proportion from each phase (l_x):

$$l_x = a_{x+1} / a_{\text{initial}}$$

Where:

$a_{(x+1)}$ = Organisms quantity in each anterior phase.

a_{initial} = Organisms quantity at beginning phase.

Life expectation (e_x):

$$e_x = T_x / l_x$$

Where:

T_x = Time left to live.

l_x = Survival proportion from each phase.

Table 1. Mean values (\pm S.D.) of *C. dubia* production fed with three experimental diets.

Día de cultivo	<i>Sphaerocystis</i> sp.	<i>Chlorolobion</i> sp.	<i>Sphaerocystis</i> + <i>Chlorolobion</i>
0	60 \pm 7	60 \pm 10	60 \pm 9
7	69 \pm 7	62 \pm 4	40 \pm 4
14	55 \pm 6	80 \pm 7	146 \pm 13
21	101 \pm 7	133 \pm 5	745 \pm 26
28	353 \pm 25	241 \pm 21	2,362 \pm 22
35	953 \pm 34	408 \pm 29	5,67 \pm 248
42	2,046 \pm 38	629 \pm 20	11,535 \pm 30
49	3,775 \pm 33	887 \pm 22	20,930 \pm 42
56	6,283 \pm 45	1,150 \pm 15	35,019 \pm 51
63	9,715 \pm 22	1,378 \pm 36	55,119 \pm 40
70	14,213 \pm 50	1,515 \pm 18	82,700 \pm 39
77	19,923 \pm 41	1,496 \pm 49	119,394 \pm 24
84	26,987 \pm 46	1,242 \pm 45	166,989 \pm 49
91	35,549 \pm 32	664 \pm 43	227,433 \pm 47

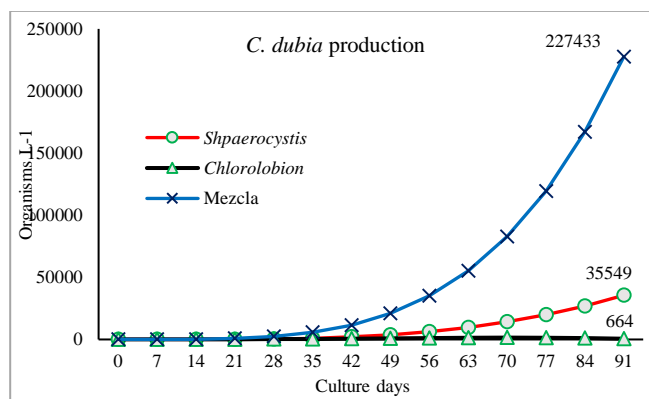


Fig. 3: *C. dubia* production with three experimental diets.

RESULTS

Ceriodaphnia dubia

Table 1 and Fig.3 show mean values (\pm S.D.) of *C. dubia* production fed with the experimental diets. The highest production was 227,433 \pm 47 org L⁻¹ (227.43 org mL⁻¹) reached at 91 days of culture, using mixed diet

(*Sphaerocystis* sp + *Chlorolobion* sp.). With *Sphaerocystis* sp. diet, density reached the highest production with 35,549 \pm 32 org L⁻¹ (35.54 org mL⁻¹), meanwhile with *Chlorolobion* sp. diet only reached 1,515 org L⁻¹ (1.51 org mL⁻¹) at 70 culture days. The tendency grow curves

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Table 2. Life table of organisms produced of *C. dubia* from each experimental diet.

Microalgae diet	Reproduction rate	Cohort generational time	Growth instantaneous rate
	$\sum lxmx$	$\sum xlxmx/Ro$	$\log_e Ro/Tc$
	Ro	Tc	r
<i>Sphaerocystis</i> sp	592	9.64	0.66
<i>Chlorolobion</i> sp.	24	5.96	0.54
<i>Sphaerocystis</i> sp. + <i>Chlorolobion</i> sp.).	3789	9.80	0.84

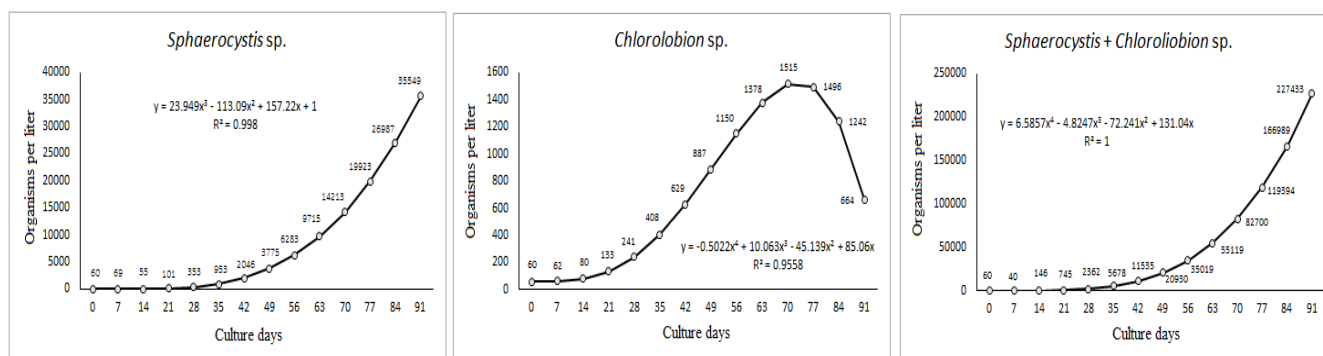


Fig. 4. Tendency curves of population growth of *C. dubia* culture fed with three experimental diets.

are shown in Fig. 4. Mixed diet and *Sphaerocystis* sp. diet, have organism production until 91 culture days, with *Chlorolobion* sp. diet, culture decrease at 70 culture days.

Tables 2 shown Life Table per experimental diet for *C. dubia*. On those tables, it can be observe that mixed diet showed a better reproduction rate per female with 3,789 org. per female and lowest value was obtain with *Chlorolobion* sp. with only 24 org. per female. With respect to GTC, diets of *Sphaerocystis* sp. and mixed showed similar values (9.64 and 9.80 days respectively). The *Chlorolobion* sp. diet has smaller GTC (5.96 days), showed lowest density production as well with $r = 0.54$, meanwhile

Sphaerocystis sp. diet showed $r = 0.66$ and for mixed diet it had an $r = 0.84$.

Daphnia pulex

Table 3 and Fig. 5 show mean values (\pm S.D.) of *D. pulex* production fed with three experimental diets, during 91 culture days. It was observe that highest production was $23,433 \pm 42$ org L⁻¹ (23.43 org mL⁻¹) with *Sphaerocystis* sp. diet. *Chlorolobion* sp. diet obtained maximum values of $13,648 \pm 35$ org L⁻¹ (13.64 org mL⁻¹). Mixed diet reached $18,384 \pm 54$ org L⁻¹ (18.38 org mL⁻¹) density.

The tendency grow curves were showed at Fig. 6. With *D. pulex* the population maintained constant during 91 culture days

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Table 3. Mean values (\pm S.D.) of *D. pulicaria* production fed with three experimental diets.

Culture days	<i>Sphaerocystis</i> sp.	<i>Chlorolobion</i> sp.	<i>Sphaerocystis</i> + <i>Chlorolobion</i>
0	30 \pm 5	30 \pm 4	30 \pm 3
7	16 \pm 4	19 \pm 9	19 \pm 8
14	115 \pm 13	112 \pm 20	123 \pm 17
21	363 \pm 11	316 \pm 24	365 \pm 37
28	823 \pm 16	662 \pm 40	787 \pm 22
35	1,551 \pm 54	1,174 \pm 46	1,429 \pm 11
42	2,604 \pm 15	1,874 \pm 14	2,326 \pm 13
49	4,035 \pm 55	2,785 \pm 21	3,512 \pm 25
56	5,897 \pm 18	3,923 \pm 42	5,020 \pm 25
63	8,242 \pm 39	5,309 \pm 48	6,878 \pm 47
70	11,118 \pm 37	6,958 \pm 21	9,116 \pm 23
77	14,577 \pm 36	8,888 \pm 29	11,761 \pm 34
84	18,666 \pm 16	11,113 \pm 31	14,842 \pm 28
91	23,433 \pm 42	13,648 \pm 35	18,384 \pm 54

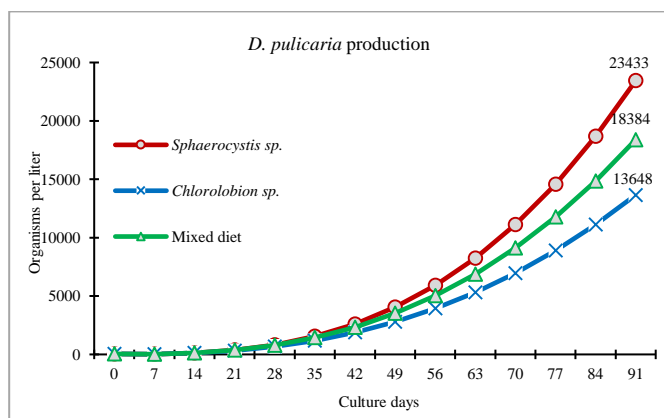


Fig. 4.- Producción de *D. pulicaria* con las tres dietas experimentales.

with the three experimental diets.

Table 4 shows Life Tables per experimental diet of *D. pulicaria* production. In that table, it can observe that *Sphaerocystis* sp. diet obtained highest production values of 781 org. per female and lowest production values with *Chlorolobion* sp. diet with 454 org. per female. With respect to GTC values, the three diets showed similar values (9.10; 8.85 and 8.95 days). With r value same thing happens (0.73, 0.69 and 0.72).

DISCUSSION

Flores-Barbosa et al. (2003) mentioned that one of principal variable that impact patterns and growth rates of different genres of cladocerans was nutritional quality and their digestibility of microalgae used for their cultivation. They found that feeding *C. dubia* with *Chlorella vulgaris* and *Scenedesmus acutus* ($0.546-1.0 \times 10^6$ cell mL⁻¹) obtained 12 org mL⁻¹. This value exceed to obtained with *Chlorolobion* sp. diet (0.664 mL org⁻¹), but not with *Sphaerocystis* sp (35.54 mL org⁻¹) and mixed diets (227.43 org mL⁻¹). With respect to *D. pulicaria*, the value is similar to the one obtained with *Chlorolobion* sp. diet (13.64 mL org⁻¹), but below with *Sphaerocystis* sp. (23.43 mL org⁻¹) and mixed diets (18.38 org mL⁻¹).

Nandini et al. (2005), who worked with *C. dubia* fed with an exclusive diet of *Chlorella vulgaris* ($1.0-1.5 \times 10^6$ cell mL⁻¹), obtained r values of 0.1 to 1.5, unlike this experiment which obtained r values of 0.54 to 0.84. Peña-Aguado et al. (2005), founded different abundance picks in culture medium of many cladocerans, when used mixed diets with microalgae and yeast. These authors mentioned that *C. dubia* grows better with *C. vulgaris* (1×10^6 cells mL⁻¹) and *Scenedesmus acutus* ($0.5 \times$

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Table 4. Life table of *D. pulicaria* organisms fed with three experimental diets.

Microalgae diet	Reproduction rate	Cohort generational time	Instantaneous growth rate
	$\sum lxmx$	$\sum xlxmx/Ro$	$\log_e Ro/Tc$
	Ro	Tc	r
<i>Sphaerocystis</i> sp	781	9.10	0.73
<i>Chlorolobion</i> sp.	454	8.85	0.69
<i>Sphaerocystis</i> sp. + <i>Chlorolobion</i> sp.).	612	8.95	0.72

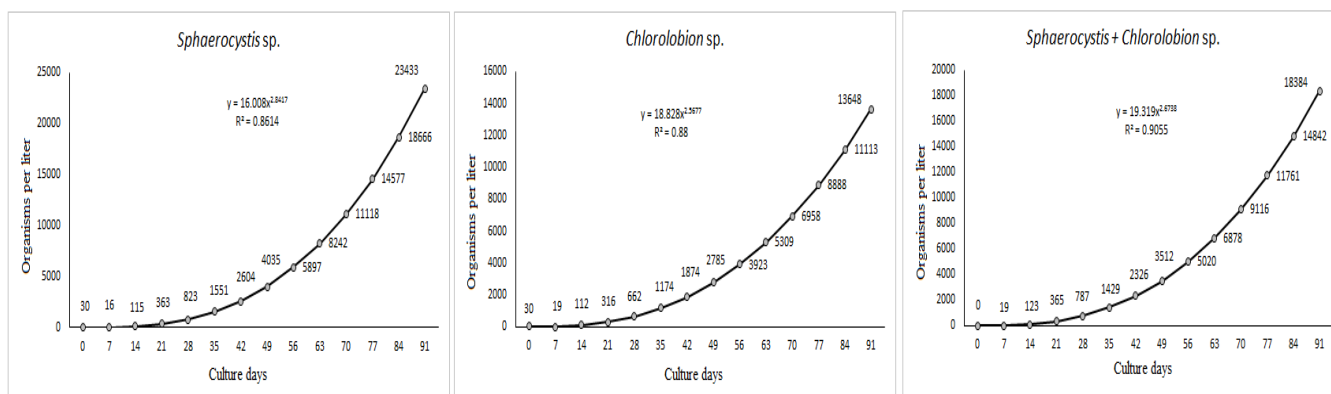


Fig. 5. Tendancy curves of growth population of *D. pulicaria* fed with three experimental diets.

10^6 cells mL^{-1}) diets. This occurs due to presence of different sizes in microalgae cells that can feed different stages of these cladocerans and their different nutritional composition, *C. vulgaris* has less lipid, proteins and carbohydrates concentration than *S. acutus*.

Nevertheless, they did not founded significant differences between the use of a single microalgae diet or mixed microalgae diets with yeast. These values are different with what we founded in this research, because mixed diet with *Sphaerocystis* sp. and *Chlorolobion* sp.

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with yeast obtained a difference of 12 org mL⁻¹. Nandini et al. (2015), mentioned that r values of specific species depends in their numerical abundance and time that it reached, thereby allowing the efficiency of determined microalgae diet or mixed diet with yeast. Alva-Martínez et al. (2007), who worked with *C. dubia* also, founded r values of 0.07 to 0.26. These authors mentioned that diet rich with *Microcystis* sp. obtained low values of reproduction rates because their nutritional deficiency.

Sarma et al. (2006), mentioned that in *C. dubia* and *D. pulex* cultured at 5 gL⁻¹ salinity concentration and fed with *Chlorella* sp. (0.25-1.5 x 10⁶ cells mL⁻¹), obtained r values between 0.34 to 0.22 range, lower values compared with this work with *C. dubia* (0.54-0.84) and *D. pulicaria* (0.69-0.73). Sarma et al. (2006), explained that most cladocerans groups did not show reproduction and survival when they are cultured up 5 gL⁻¹ salinity concentration. Savas and Erdogan (2006), founded in *C. quadrangula* fed with *Scenedesmus acuminatus* (15-75 x 10⁴ cell mL⁻¹) densities of 8.63 at 20.10 org mL⁻¹ with an r = 0.199-0.237. These values were lower with respect to obtained data with *C. dubia* and *D. pulicaria* fed with *Sphaerocystis* sp. (23-35 org mL⁻¹; and r = 0.66-0.73). Sarma et al. (2006), founded that low concentrations of food in culture medium shown significant differences in cladocerans densities, with respect to those cultures with high food concentrations. High food concentration in culture medium allow to different cladocerans populations shown high grow rates, comparing to culture mediums with limited food input.

Gama-Flores et al. (2007), founded an r = 0.342 in *C. dubia* cultures fed with *Chlorella* sp. (0.5 x 10⁶ cells mL⁻¹), lower values of r, with respect to founded in this experiment with 0.54-0.84 range for *C. dubia* and for *D. pulicaria* of

0.69 to 0.73 range. Sanchez-Ortiz et al. (2010), founded r values of -0.12 to 0.14 with *C. dubia* fed with *Scenedesmus acutus* (0.5 x 10⁶ cells mL⁻¹) and organisms density in 0.2 to 6.0 org mL⁻¹ range. These values are below the values founded in this work with *C. dubia* and *D. pulicaria* fed with *Sphaerocystis* sp. and *Chlorolobion* sp. (5 x 10⁶ cell mL⁻¹). Bear out that microalgae mixed diets complemented with yeast, increase cladocerans populations grow rate. Fernandez et al. (2012), mentioned that a microalgae mixed diet, complemented with cyanobacteria improve growth and reproduction not only in cladocerans, but only in rotifers. These authors and Perez-Morales et al. (2014), also mentioned that cyanobacteria used as only food can produce density decrease due to their poor nutritional quality and poor feeding efficiency for cladocerans.

Pietrzak et al. (2013) mentioned when cultures of different species of cladocerans in same container are cultured, it must be consider the different sizes from each cladocerans groups and the microalgae cell sizes used to feed them, because of their competition between small species with bigger size species. Alcántara-Azuara (2014), who worked with *D. pulex* fed with *Haematococcus pluvialis* and *Chlorella vulgaris* mentioned that increase microalgae concentration in culture medium does not ensures the increase of cladocerans production, because it can cause a loss of female fertility due to intraspecific competition for available space. These authors mentioned that supply of brown microalgae (diatoms) rich in lipid and carbohydrates, improves digestive capacity of *D. pulex* improving females reproductive rates and consequently an increase in density crops.

Conde-Porcuna et al. (2014), explained that one physical variable that need to be consider carefully was photoperiod, which includes in ehippia production, when sexual

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reproduction is present in these organisms but not also for alteration of other factors like water temperature, depredation risk or dense dependent factors like food availability and overcrowding. This problem with photoperiod did not show when cladocerans reproduce in a parthenogenetic form. *D. pulicaria* presents ephippia productions when food concentration or nutritional quality are low.

Sikora et al. (2014), who worked with *D. pulicaria* fed with *Scenedesmus obliquus*, in photoperiod cycles of 16:8 hours light/darkness, founded that grow rate decrease when culture medium was at a temperature of 32°C unlike cultures in temperatures of 16-24°C at same light/darkness conditions. These authors mentioned that photoperiod variable affects more in bigger sizes cladocerans unlike smallest sizes bodies like *D. cucullata*. The differences founded in grow rates relative to size body must be determined for their phosphorous demand, which are greater in bigger species unlike smallest species. The phosphorous demand in cladocerans species is inversely proportional in body size when the species reach sexual maturity. The smallest species needs a bigger quantity of phosphorous per biomass unit than bigger body species. William et al. (2015), mentioned that cladocerans body sizes join in greater or less degree of interspecific competition. This size efficiency hypothesis (bigger zooplankton is a best competitor), often does not impact in all cladocerans species to obtain their food, but positive way for increasing fertility in populations and therefore a production increase in biomass stocks.

These all considerations allow obtain better laboratory cladocerans productions for their use in teaching, scientific researches and massive productions to use in aquaculture or aquariophilya industries.

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