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Laboratory controlled production of *Lepadella* sp. (Bory of St. Vincent, 1826), using three different microalgae and their combination, enriched with yeast

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Abstract
The present study was made with *Lepadella* sp. rotifer using four experimental diets (*C. vulgaris, H. pluvialis, Haematococcus* sp. and equal mix of these), for 60 culture days. Microalgae were maintained at a concentration of 0.5-1.0 x 10^6 cell mL^{-1}, pH of 7-8 and temperature of 25±2°C. The diet that show better organism density was *H. pluvialis* with 1,870,727±125 org $20L^{-1}$, while *Chlorella* sp. diet showed the lowest density with 550,572±128 org $20L^{-1}$. All diets showed significant differences ($P<0.001$) among them, regarding to their final densities. Ro value ranged between 30 and 93 new individuals per female. *H. pluvialis* showed a Ro=93 org per female and an r=0.25. All diets presented a Tc value between 17.31 and 18.44 and a value of r between 0.18 and 0.25. This experiment proves that it is possible to manage the culture density of this rotifer, depending producer needs.

Keywords: laboratory, production, *Lepadella*, microalgae, enriched

1. Introduction
Rotifers is one of the more used zooplanktonic group as live food in the early stages of feeding offspring of fish and crustaceans of both freshwater or marine origin [1, 2], because of their size (100-340 µm), nutritive value and its high reproductive rate, allow to produce them in massively form [3, 4].
At intensive rotifer production, the first drawback to overcome is to get the proper food. Microalgae species like: *Nannochloris oculata, Chlorella* spp, *Isochrysis galbana, Tetraselmis suecica, Phaeodactylum tricornutum* have been used, because they have an important role in its development and growth, as well as the use of yeast *Saccharomyces* sp. [5]. It has been proved that green microalgae are rich in carbohydrates and diatoms have more lipids, which are exploited by the organisms in culture, for which they are considered as a complement and not a substitute, because its appropriate use increases the survival, development and growth of the aquatic organisms fed with them [6]. Hirayama and Funamoto [7], recommends the use of yeast as food because it provides high population densities at low production costs. Even though it has been reported that the use of yeast provides low nutritive value to rotifers [8], an alternative to improve their quality is to enrich them by suspending them in a microalgae culture during six hours before being supplied as food to fish and crustaceans larvae [9].
One of the main families of Rotifera phylum is Lepadellidae [10], being the genera *Lepadella patella* one of the most used because of its small size (103 µm mean value), which can be use in larviculture of fish and crustaceans because they do not have problem with mouth diameter. It has been observed that *L. patella* maintain an adequate culture density when the density of microalgae *Chlorella* sp. maintains a minimum density of 1.0 x 10^6 cells mL^{-1}, which increases until reaching a maximum of 5.0 x 10^6 cells mL^{-1} [11].
Aim of this study was to culture *Lepadella* sp. using the unicellular microalgae *Chlorella sp.*, and others like *Haematococcus fluvialis*, *Spherocystis* sp., and their combination to determine the highest density in culture media in laboratory.

2. Materials and Methods
2.1 Obtainment of rotifer population
Water samples of 20L were obtained from the culture ponds in Centrode Investigaciones

Bilógicas de Cuemanco (CIBAC) Mexico City, which was filtrated through a sieve of 50 µm to eliminate residuals and unwanted zooplanktonic organisms. The water sample was taken to the Laboratory of Live Food Production (LLFP) at Universidad Autónoma Metropolitana Unidad Xochimilco (UAM-X) of El Hombre y su Ambiente Department (DEHA) and were placed in 20 L containers with continuous aeration and light for 12 hours (white light bulb of 40W). Were maintained at a temperature of 25±2°C and pH between 7-8. They were fed with a combination of three microalgae (*Chlorella vulgaris, Haematococcus fluvialis* and *Spherocystis* sp.) in equal parts, for 21 days to observe the presence of rotifers and easily separate the ones that belong to genus *Lepadella* sp.

2.2 *Lepadella* sp. rotifer separation

During the first 21 days the rotifers were separated manually with the aim of a stereoscopic microscope Olimpus SZ40. Organisms were placed in Petri plates (10cm of diameter) with 10 mL of water and 1mL of combined microalgae food. When the organisms reached a density of 2 org mL^{-1}, were placed in a flask of 500 mL and so on until reaching a culture volume density of 10 L.

2.3 Experimental design

Plastic containers of 20 L were prepared (original and two replicas), with 12 hours of light and continuous aeration; a temperature of 25±2°C and a pH between 7-8. From the 10 L stock culture container, the organisms were concentrated in 1 L of water and 10 samples of 100 µL were taken to obtain the mean density per milliliter, and therefore place in each culture container an initial population density of 1 org mL^{-1}.

To each used diet: 1) *C. vulgaris*; 2) *H. fluvialis*; 3) *Spherocystis* sp. and 4) Combined diet (equal parts), it was added *ad libitum* 500 mL of microalgae culture at a minimum concentration of 0.5-1.0 $x10^6$ cell mL^{-1}. Each third day, it was supplied to each diet 2 mL of dry active yeast at a concentration of 2 g of yeast in 4 L of water.

2.4 Sampling

Each third day, ten samples of 1 mL were taken from the culture container to determine the population density (±S.D.). The data was extrapolated to 1 L of culture. Density values were considered until the culture presented a lack of growth or organisms' death.

2.5 Information processing

Obtained data were introduced to a spreadsheet in the program Excel 2013 to obtain the average value of density and its standard deviation (±S.D.). To determine the survival percentage the next formula was used:

$$\text{Survival} = \frac{(a_{x+1})\ x\ 100}{a_x}$$

Where:

a_x = Phase n organism density

a_{x+1} = Organism density inthe next phase

Reproduction rate = R_o

$$\sum l_x m_x$$

Where:

$l_x m_x$ = Organismos producidos por cada individuo original en cada fase

Generation time of the cohort = T_c

$$\sum x\ l_x m_x / R_o$$

Where:

x = Time

$l_x m_x$ = Produced organisms per each original indiviual in each phase

R_o = Reproduction rate

Intrinsic growth rate = r

$$Log_e R_o / T_c$$

Also, it was determined the growth tendency curves of each population using the program Excel 2010.

3. Results

The culture had a duration of 60 days. Mean values of each sampling (each third day), and its standard deviation, are presented in Table 1. The highest density was found with microalgae *H. pluvialis* with 1,870,727 ±125 organisms L^{-1} of culture (1,871 org mL^{-1}). The lowest density values were obtained with microalgae *Chlorella* sp. with 550,572 ±128 organisms L^{-1} (550 org mL^{-1}). The other two diets (mixed diet and *Spherocystis* sp.) were between 621-1,000 org mL^{-1} respectively. Variance analysis (ANOVA) showed significant differences ($p<0.001$) between all diets (Fig. 1).

Growth tendency curves of the *Lepadella* sp cultures with their respective formula are shown in Fig. 2. All tendency curves were logarithmic, with a $R^2= 0.98$.

Regarding to reproductive variables (Table 2), the diet with microalgae *H. pluvialis* showed the highest value with 93 organisms produced per female, while the lowest value was the diet with *Chlorella* sp. with 27 organisms per female in each laying. The other two diets had values between 30-49 org per female (mixed diet, *Sphaerocystis* sp.). Regarding to generational time of the cohort, all diets presented similar values (17-18 days). For the growth rate (r), the highest value was 0.25 with *H. pluvialis* diet and the lowest with mixed diet with 0.18. While the diets *Chlorella* sp. and *Sphaerocystis* sp. obtained values between 0.19-0.21 respectively.

4. Discussion

One of the main factors for an adequate rotifer culture is the used food, and its concentration. Kostopoulou and Vadstein [12], mentioned that addition of 0.4 to 0.5 mg C L^{-1}, was the adequate range of food (microalgae or yeast), to obtain growth rates (r) of 1.57±0.07. This has been observed in rotifers species of genus *Brachionus* sp. like: *B. plicatilis*, *B. Nevada* and *B. cayman*, which were fed with microalgae *N. oculate*, in which it was observed an increase in growth rate when food quantity is above 4.0 mg C L^{-1}.

The cultures made with *B. angularis* fed with microalgae *Chlorella* sp. and enriched with yeast, obtained densities of 30 to 60 org mL^{-1} [13], unlike with *Lepadella* sp. in this experiment where it was obtained a density of 550 org mL^{-1}. With the combined diet and *Sphaerocystis* sp. it was obtained a range of 621-1000 org mL^{-1} respectively, while *H. pluvialis* reached a density of 1,871 org mL^{-1} and growth rates (r)

between 0.19-0.27. Nandini and Sarma [11], that worked with *Lepadella patella* fed with *C. vulgaris*, in densities of microalgae cells between 0.25 to 4 x10^6 cell mL^{-1} during 22 days of culture, at 25°±2°C, obtained densities of 1,000 ±96 org mL^{-1}, with values of r=0.35, using a food concentration of 1.0 x10^6 cell mL^{-1}, superior values to the ones found in this experiment with only 550 org mL^{-1} with *Chlorella* sp. and 621 org mL^{-1} with the combined diet, being almost the same food concentration (0.5 and 1.0 x10^6 cell mL^{-1}), similar with *Sphaerocystis* sp. (1,000 org mL^{-1}), but below with *H. pluvialis* diet (1,871 org mL^{-1}). These authors mention that to obtain a good zooplanktonic production three conditions must be given: a) The food was available all the time, b) an adequate temperature, and c) interaction with other species. In this study the first two requirements were met, in the case of the third one the culture was made only with *Lepadella* sp. These authors mention that the relation of the rotifer size used in the culture and the food concentration is important, because big species require more food than small species. Also, in big rotifer species, the high microalgae concentration in the culture medium inhibit its growth, unlike small species that need high concentration of microalgae. In the case of *Lepadella* sp. these authors mention that interaction with other species of rotifers give better results on its growth, as they demonstrated using *Philodina roseola*.

Nandini *et al*. [14], that worked with *L. patella* fed with *C. vulgaris* at concentrations of 0.4 x10^6 cell mL^{-1} obtained densities of 738 org mL^{-1} starting its cultures with only 5 org mL^{-1}, superior values to the ones in this experiment where the culture started with 20 org mL^{-1} with the same microalgae, as with the combined diet (621 org mL^{-1}), but below the obtained values with *Sphaerocystis* sp. and *H. pluvialis* diets (1,000 and 1,871 org mL^{-1}resepctively). These authors mention that the used food for rotifers culture must cover the minimum nutritional requirements for their metabolic needs. It is important to consider that to determine the minimum quantity of food concentration, either microalgae or yeast, these most maintain an r=0, therefore *L. patella* needs a minimum concentration of 0.025 x 10^6 cell mL^{-1} to obtain r values superior to zero. In this experiment it was considered to maintain concentrations between 0.5-1.0 x 10^6 cell mL^{-1}. These authors mention that *L. patella* grows better with cell concentration of 1.0 x 10^6 and above this concentration it dies when microalgae *C. vulgaris* is used. It must be said that in rotifers culture there is an inverse relation regarding the concentration of food and the rotifer size, because a higher concentration of food is better for a culture of small rotifers and the bigger rotifers must be cultured with a lower food concentration. But for this, it is necessary to make studies with microalgae and the different rotifer species to find the adequate food for each specie [14, 15]. Ajah [15], mentioned that size, shape and mobility of used phytoplankton in rotifer culture can modify the nutritional behavior of organisms and therefore the success of its massive production.

Madhu *et al*. [16], cultured the marine rotifer *Colurella adriatica*, which belong to Family Lepadellidae, obtained densities of 1,000 org mL^{-1} using microalgae *Nannochloropsis oculate*. By using the same microalgae, but enriching the culture medium with yeast, the density decreases to 950 org mL^{-1} in 14 culture days, higher values are obtained when it is grown with only yeast (650 org mL^{-1}). Similar values to the ones found in this experiment with diets of *Chlorella* sp., combined and *Sphaerocystis* sp. with 550, 621 and 1,000 org mL^{-1} respectively.

Table 1: Mean values (±S.D.) of obtained densities of *Lepadella* sp. cultured with the four experimental diets, enriched with yeast.

Sampling	*Chlorella sp.*	*H. pluvialis*	*Sphaerocystis sp.*	Combined
0	20,000	20,000	20,000	20,000
3	100,978±102	180,489±375	70,800±189	50,778±164
6	140,169±97	340,789±373	200,281±178	130,254±77
9	210,460±97	610,720±355	340,371±183	210,949±168
12	260,633±89	800,828±378	440,367±188	280,118±126
15	300,645±140	950,649±345	520,121±196	320,903±87
18	330,923±106	1,070,759±355	580,457±195	360,813±101
21	360,695±128	1,170,997±366	630,813±200	400,119±125
24	390,096±117	1,260,867±340	680,454±186	420,983±120
27	410,214±125	1,340,690±367	720,546±190	450,508±166
30	430,108±108	1,410,688±368	760,208±192	470,768±103
33	440,822±131	1,480,018±361	790,519±188	490,812±166
36	460,387±102	1,530,797±342	820,543±178	510,678±162
39	470,826±93	1,590,114±349	850,324±201	530,394±141
42	490,158±102	1,640,036±378	870,900±185	540,984±130
45	500,399±122	1,680,619±350	900,297±181	560,463±171
48	510,560±134	1,720,905±345	920,540±190	570,847±157
51	520,650±108	1,760,932±338	940,646±195	590,147±126
54	530,677±119	1,800,728±352	960,633±201	600,373±162
57	540,650±157	1,840,320±134	980,511±190	610,532±345
60	550,572±128	1,870,727±125	1,000,294±366	620,632±200

Table 2: Reproductive values of *Lepadella* sp. cultured with four different microalgae diets, enriched with yeast.

Experimental diet	Reproduction rate (Ro)	Cohort generational time (Tc)	Growth rate (r)
Chlorella sp.	27	17.31	0.19
H. pluvialis	93	18.42	0.25
Sphaerocystis sp.	49	18.44	0.21
Combined	30	18.43	0.18

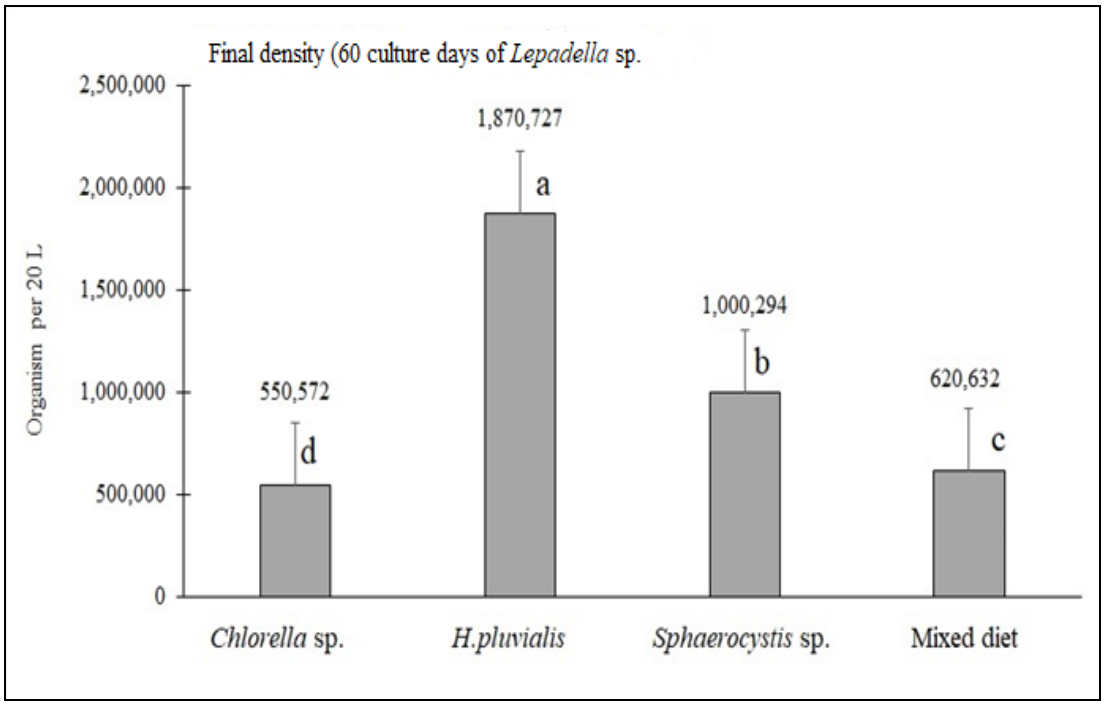

Fig 1: Final density of *Lepadella* sp organism fed with the four experimental diets enriched with yeast

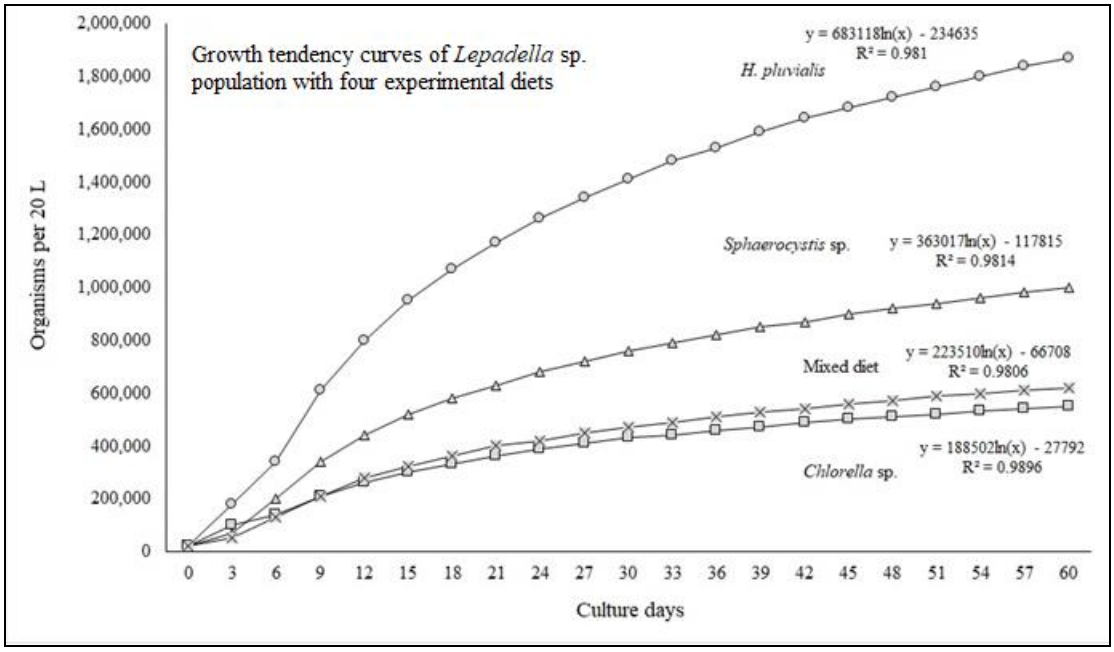

Fig 2: Growth tendency curves of *Lepadella* sp. fed with the four experimental diets enriched with yeast

5. Conclusion

Whit this experiment we hope to contribute to the knowledge of this rotifer culture, using different sources of microalgae, so that in the end, it is the researcher or producer the one who decides, according to their necessities or management, the diet to be used and therefore maintain continuous production or only maintenance cultures for not losing the strain.

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