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Population density comparison and reproductive potential of *Daphnia pulicaria* (Forbes, 1823) fed with chlorophytes (*Scenedesmus* sp + *Chlorococcum* sp.) and diatoms (*Pinnularia* sp.)

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Abstract
Present work was made to know population growth of *Daphnia pulicaria* fed with four experimental diets with chlorophytes and diatoms microalgae during 60 culture days in 200 L plastic beakers with an initial density of 500 organisms. Used experimental diets were: 1) Chlorophytes 100%; 2) Diatoms 100%; 3) Chlorophytes 25%, Diatoms 75%; and 4) Chlorophytes 75%, Diatoms 25%. With Chlorophytes 100% diet the organisms died at the sixth culture day. Diatoms 100% obtained the highest density with 18,221 org 160 L^{-1}, meanwhile, the lowest density was in diet Chlorophytes 75% and Diatoms 25% with 10,761 org 160 L^{-1}. Ro variable fluctuated between 3,443-5,869 per female. Tc variable fluctuated between 16.30-16.49; days and r variable fluctuated between 0.50-0.53. ANOVA test showed significant differences ($P<0.05$) between all densities from the four experimental diets. In *D. pulicaria* culture case it is recommendable the use of diatoms for their optimal growing in laboratory systems.

Keywords: *Daphnia pulicaria*, chlorophytes, diatoms, population density, life table

1. Introduction
Cladocera group is constituted by freshwater crustaceans, commonly named "water flies" because of their small size and movements in water [1]. This common group of small crustaceans have some characteristics that make them fit for their use in aquaculture, like their small size, short life cycle, fast growing and consequently early maturity that allows them to produce a lot offspring's [2].
Daphnia gender consists approximately of 100 species from which the best know are: *Daphnia magna*, *Daphnia pulex*, *Daphnia longispina*, *Daphnia pulicaria*. These organisms feed with particles suspended in water, that is why they are called filter feeders and they show different responses and sensitivities to different diets [3].
D. pulicaria is commonly founded at the bottom of water bodies and it is more abundant in the spring [4, 5]. Their reproduction is parthenogenetic and only when water conditions are adverse, they present sexual reproduction. The adult organisms produce resilient eggs which can resist dry and freeze season until water conditions are favorable to hatch them [6, 7]. As organisms that feed through filtration, their main food can be bacteria and unicellular microalgae.
The microalgae are considered as functional food because they can increase, in a diet, their nutritional content because they have high carbohydrates, lipids, vitamins, minerals, polyunsaturated fatty acids (w-3, w-6), and antioxidants (carotenes). Someone has a high amino acids content like *Scenedesmus* sp., which have higher levels than FAO patter, and have high proteins levels between 25 to 65% [8, 9]. *Chlorella* sp. and *Scenedesmus* sp. microalgae are commonly used to make great variety of zooplankton culture, including *Daphnia* gender [10].
An important group of microalgae and which has not been studied was diatoms which have high lipid content. Cladocerans culture systems improve their density production when diatoms were incorporated to food source, because they improve the digestibility capacity of organisms of *D. pulex*, also their nutritional content was higher in lipids and carbohydrates when diatoms were added to culture system with respect to unicellular green microalgae [11]. These authors also mentioned that diatoms food source increase the reproductive rate of *D. pulex* females, and consequently a fast growth to reach sexual maturity sooner and began to reproduce and so regenerate the population density in culture media.

Because of the above, the goal of this research was to determine the population density and their reproductive potential of *D. pulicaria* fed with chlorophytes and diatoms in different concentrations to obtain better results in biomass production in laboratory conditions.

2. Materials and Methods

2.1 Obtaining organisms

D. pulicaria organisms were obtained from a 20 L water sample obtained at Centro de Investigaciones Biológicas y Acuícolas de Cuemanco (CIBAC). The sample was taken to Life Food production Laboratory at Universidad Autónoma Metropolitana Xochimilco and filtered through 20 µm mesh. The organisms were placed in 1 L of water and observed in Optical Microscope Leica ICC50 HD (10 and 20x). The cladoceran specie was identified with Zooplankton Image Key (V.5.0) program.

Five adult organisms were placed in a Petri dish (10 cm diameter) with 20 mL of water and 2 mL of microalgae (25 replicates). When organism's density reached 2 org mL^{-1}, were placed in 1 L of culture medium and later placed in 20 L culture medium when again the culture density reached 2 org mL^{-1}.

2.2 Experimental design

Twelve plastic beakers of 200 L capacity were filled with 160 L of water, with continuous light and air supply (Fig.1). Four experimental diets were tested: 1) Chlorophytes 100%, 2) Diatoms 100%; 3) Chlorophytes 25% and Diatoms 75%; and 4) Chlorophytes 75% and Diatoms 25%. Each diet was made by triplicate. Every third day, three samples of 10 mL were taken, and all organisms were counted to obtain a mean value and their standard deviation (±D.S.).

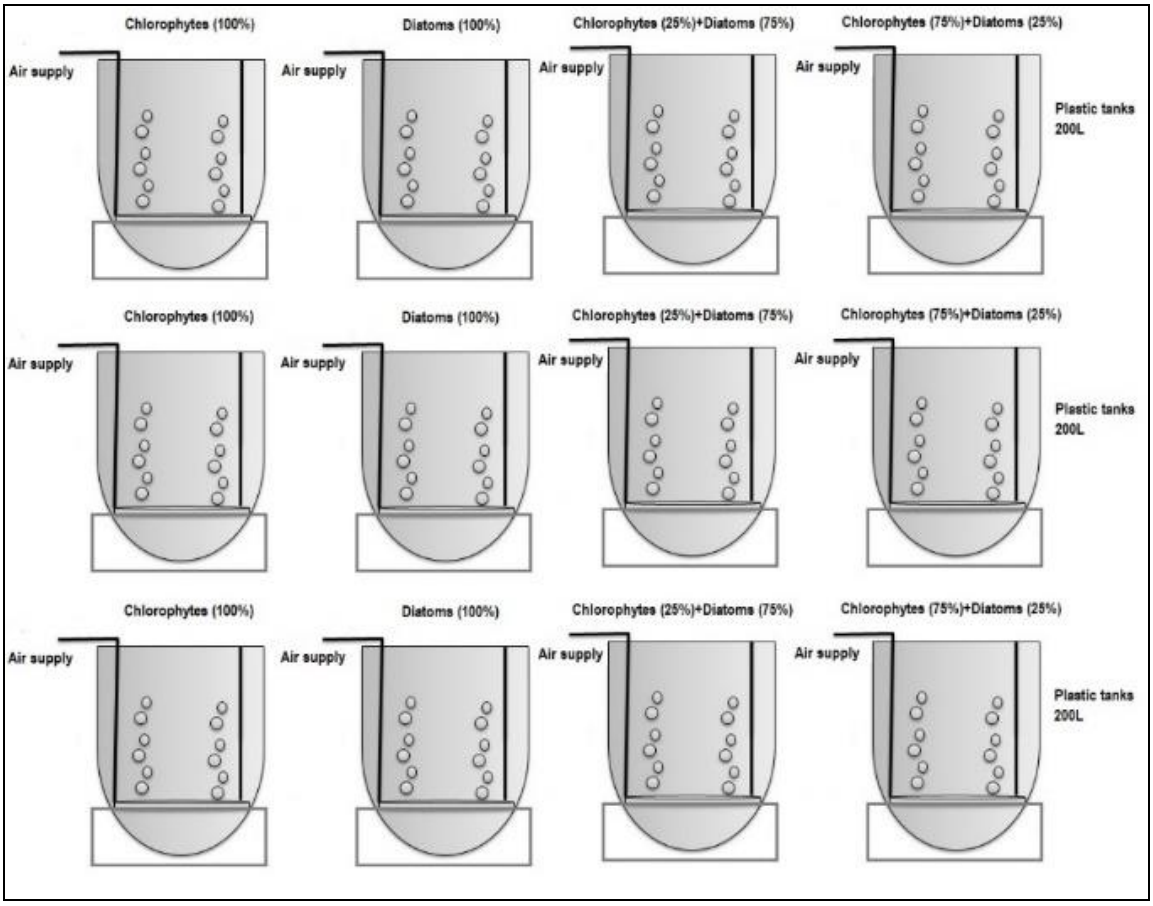

Fig 1: Experimental design used to culture *D. pulicaria* using four experimental diets with microalgae (Chlorophytes and Diatoms).

2.4 Processing data

Each sampling value per experimental diet were introduced in a data base in Excel 2010 to obtain descriptive analysis. The values were extrapolated to 160 L. Also, the density tendency curves for each diet were obtained.

Density values at each sampling day were introduced in a Life Table Program made in Excel 2010 to obtain the following reproductive parameters:

Reproduction rate: $Ro = \sum l_x * m_x$

Where:

$\sum$= summation

lx = survival proportion in each life phase.

mx = organisms produced by each live organism in each life phase

Intrinsic Growth Rate: $r = \log_e R_o / T_c$

Where:

$\log_e R_o$ = logarithm of natural reproduction rate

T_c = cohort generation time

Cohort generation time: $Tc = \sum x * lx * mx / Ro$

Where:

$\sum$= summation

lx = survival from each phase

mx = organisms produced at each phase

Ro = reproduction rate

2.5 Statistical analysis

ANOVA test was made to obtain significant differences ($P<0.05$) between density culture medium from each experimental diet. When ANOVA test showed significant

differences a multiple mean comparison analysis was made using Tukey test. In both cases SYSTAT 13.O Program was used to make the analysis.

3. Results

Table 1 shows density mean values (±S.D.) of *D. pulicaria* in the four experimental diets from each sample day. Organisms fed with Chlorophyte 100% diet, died at sixth day. This experimental diet was made three times with same result. Highest densities were found in Diatoms 100% diet with 2,915,349±288 org 160 L^{-1} and Chlorophyte 25% and Diatoms 75% diet with 2,614,758±186 org 160 L^{-1}. ANOVA and Tukey tests showed significant differences between four experimental diets ($P<0.05$).

Table 1: Mean values (±S.D.) of population density of *D. pulicaria* fed with four experimental diets in 160 L culture system in laboratory.

Sampling day	Chlorophyte (100%)	Diatoms (100%)	Chlorophyte (75%) Diatoms (25%)	Chlorophyte (25%) Diatoms (75%)
0	500±35	500±32	500±36	500±33
3	800±29	5,388±177	3,671±121	9,493±278
6	0	21594±168	12,810±337	30,681±270
9		36,351±340	21,513±115	50,852±268
12		46,687±153	28,316±198	66,298±284
15		51,106±331	32,541±326	74,852±139
18		49,591±120	34,292±260	75,887±142
21		43,606±230	34,459±329	70,316±276
24		36,090±123	34,710±207	60,590±219
27		31,463±326	37,501±160	50,703±129
30		35,624±202	46,070±330	46,188±126
33		55,949±287	64,436±187	54,116±123
36		101,293±290	97,404±172	83,102±271
39		181,991±120	150,563±229	143,297±200
42		309,855±139	230,281±335	246,394±195
45		498,177±117	343,713±105	405,626±187
48		761,726±257	498,796±89	635,766±147
51		1116,751±155	704,250±194	953,127±112
54		1,580,980±176	969,580±325	1,375,560±159
57		2,173,618±161	1,305,072±200	1,922,459±263
60		2,915,349±288[a]	1,721,796±245[b]	2,614,758±186[c]

Fig. 2 shows tendency curves of population density increase of *D. pulicaria* at four experimental diets. The tendency curves were best shown with a polynomial grade three curves.

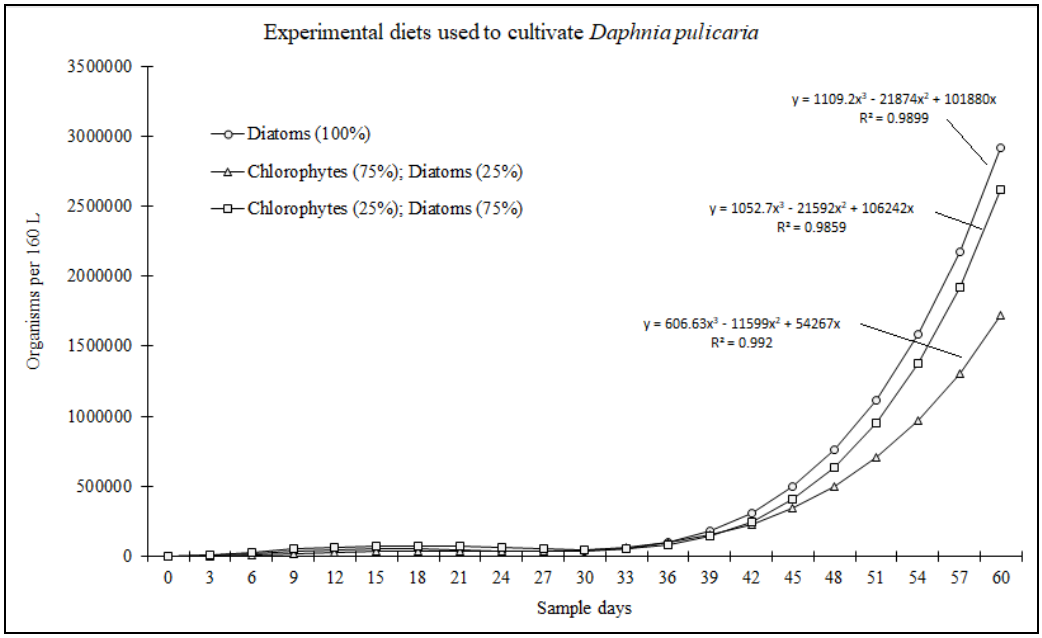

Fig 2: Tendency curves of population density increase of *D. pulicaria* fed with experimental diets.

Reproductive values are shown in Table 2. Tc and r values presented similar values (Tc=16.30-16.49; r=0.50-0.53). Different values were shown with Ro values in Chlorophyte 75% and Diatoms 25% diet. We can observe a difference of 2,000 organisms produced by females. Diatoms 100% and Diatoms 75% and Chlorophyte 25% diets show Ro values of 5,288-5,869 org hembra^{-1} respectively.

Table 2: Reproductive values of *D. pulicaria* population fed with experimental diets.

Experimental diets	Reproduction rate per female (Ro)	Cohort generation time (Tc)	Population increase rate (r)
Chlorophyte (100%)	0	0	0
Diatoms (100%)	5,869	16.49	0.53
Chlorophyte (25%); Diatoms (75%)	5,288	16.47	0.52
Chlorophyte (75%); Diatoms (25%)	3,443	16.30	0.50

4. Discussion

Short term studies related with zooplankton food selection provide information about microalgae preferences for some zooplankton specie. Nevertheless, this does not improve a positive density growth with exclusive microalgae diet [12], and microalgae species can vary with different culture condition, but a mixed carefully selection of microalgae can offer an excellent nutritional value diet to feed fish and crustacean larvae stage [13, 14].

In this study, the production analysis of four experimental diets showed that Diatoms 100% was highest productive with a maximum density of 2,915,349±288 org 160 L^{-1}. This can be explained because diatoms have a high lipid content necessary to zooplankton growth [15]. Choi *et al* [16] founded that diatoms in zooplankton food increase the food assimilation of *D. magna.* Volkman *et al*. [17], mentioned that diatoms microalgae have high content of polyunsaturated fatty acids source, specifically eicosapentaenoic acid (20:5 w-3) (7-34% of dry weight from microalgae cell). Ravet *et al*. [18], proved that lipid deficiency in zooplankton feeding affect density growth rate of *Daphnia* sp. This confirm the findings of this study, where Diatoms 100% and Diatoms 75% and Chlorophyte 25% obtained the highest density content in 160 L culture medium.

Although Chlorophyte 100% diet did not show good density results, the mixed source of Diatoms 75% and Chlorophyte 25% showed good results in *D. pulicaria* cultures in laboratory. This suggest that nutritional contribution with those two microalgae sources at that proportion percentage, increase the nutritional value of this diet.

Gunner *et al* [19] and Ahlgren *et al* [20] mentioned that *Scenedesmus* sp. is a good lipidic source in cladocerans cultures, because increase their nutritional value and diminish effect of food absence [21] also, their fatty acid profile was appropriate to *D. pulicaria* culture in laboratory [22, 23]. This was demonstrated in this study with Chlorophyte 100% diet (low concentration of lipidic content) that all population died at sixth culture day. While Diatom 100% diet improve their production when were used in *D. pulex* combined with carbohydrates, it can improve diet digestibility, increase reproduction rate per female and reduced time that females reach sexual maturity and began reproducing parthenogenetically [11, 22, 23].

A principal factor that control the increase biomass rate during long periods is food quality. This allow growth, longevity, offspring's, and other changes in life cycles characteristics in zooplankton culture systems [24]. Therefore, food quality modifications can be used as experimental strategies to produce changes in secondary production of zooplankton culture systems. In this way, is probably that chemical composition of food particles is the most important variable. But it is necessary to consider other things like cellular wall digestibility and cellular size, important variables for density growth rates from each zooplankton population. That is why is important to know not only how different microalgae size were used to specific zooplankton group, but also a limited microalgae species with specific nutritional quality were adequate to one zooplankton specie to other [24].

In cladoceran group it is important to know that food pass through intestinal tract quickly and this characteristic cannot allow optimal absorption of nutrient [25]. That is why is important to know not only the size of microalgae, but also their assimilation by the zooplankton group. Because their absorption capacity is crucial for density population in *Daphnia* sp. culture system. Cladoceran groups need that microalgae source can be assimilated completely. This was confirmed by Ahlgren *et al*. [20] which reported that microalgae quality in size and adequate nutrition content can modified density growth in cladoceran cultures systems.

Peña-Aguado *et al*. [26], mentioned the same thing with respect to growth rate (r). This variable shows the efficiency of specific diet with respect to density and offspring per female. This was demonstrated in this study with the three diets that reached values up to 0.50. Specifically, the Diatoms 100% diet who reached an r= 0.53, a reproduction rate of Ro= 5.869 per female 160 L^{-1}, and Tc value of 16.49 days. This allows that females produce more organisms than deaths and population regenerated in short time with respect other diets and therefore best productive diet. Studies with *D. dubia* [27] fed with *Chlorella* sp. (0.5 x 106 cel mL^{-1} concentration) obtained values of r = 0.342. This value was below our findings.

D. pulex studies [28] fed with *Haematococcus pluvialis* and *Chlorella vulgaris*, mentioned that it is not important to increase microalgae concentration in culture medium to obtain better results. It is better to supply a high nutritional quality and easily digestibility microalgae to obtain better results. In many cases, the increase in food concentration in culture medium of microalgae can result in female fecundity loose because of the intraspecific competency by food and space in culture medium. That is why is important to supply a diatom diet rich in lipids and carbohydrates to improve the digestibility in *D. pulex* and improve the female reproduction rate and consequently the biomass increase in culture system.

The highest population density in *D. pulicaria* culture in this study was found in Diatoms 100% diet with 2,915,349±288 org 160 L^{-1} (18 org mL^{-1}), higher values with respect studies made with *Daphnia* sp. [29] cultured with final density of 0.34 a 0.49 org mL^{-1} fed only with green microalgae.

Jong *et al*. [30], mentions that Cladocera cultures are improved in its production when used microalgae is a diatom instead of a green microalgae, which improve the digestive capacity of *D. pulex*, also its nutritional content is better in lipids and carbohydrates and increases the reproduction rate of females, as well as, a lower time lapse to reach sexual maturity and begin to reproduce to regenerate the population in culture mediums.

At last, it is important to remark that cladoceran culture is a multi-factorial problem: species type, collected sample, environmental conditions, microalgae diet, bacteria source diet, food concentration, quality of food, eater characteristics, among others, but is necessary to make laboratory experiments, principally with food type, concentration, and nutritional quality necessary to obtain better biomass sources that are used to feed larvae stage of fishes and crustaceans in aquaculture and aquarium-hobby [31].

5. Conclusion

According to the obtained results, Diatoms 100% diet in *D. pulicaria* culture can provide good population density in laboratory conditions but it is necessary to make a mixed diets with green microalgae to satisfy their nutritional requirements, like the tested diet in this work with Diatoms 75% and Chlorophytes 25%.

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