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Population density of *Daphnia* sp fed with *Scenedesmus* sp and *Pinnularia* sp supplied with yeast in laboratory conditions

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Abstract
This research shows the population growth results of *Daphnia* sp. fed with three experimental diets using the microalgae *Scenedesmus* sp and *Pinnularia* sp supplemented with yeast for 60 days in 140 L of water container inoculated with 500 organisms. The experimental diets were: 1) *Pinnularia* sp + *Scenedesmus* sp + yeast; 2) *Pinnularia* sp + yeast; and 3) *Scenedesmus* sp + yeast. The results showed that *Daphnia* sp fed with diet 1 shows the highest population density with 2,903,714 organisms in 140 L, while the lowest population density was obtained with diet 3 with 2,208,205 organisms. The variable Ro fluctuated between 4, 380 to 5, 718 per female. The variable Tc remained stable over the 16 days and the variable r fluctuated between 0.37-0.58. The analysis of variance showed significant differences ($p<0.05$) among all the densities of the three experimental diets. The diet composed of diet 1 is recommended over diets 2 and 3.

Keywords: *Scenedesmus* sp, *Pinnularia* sp, *Daphnia* sp, yeast, population density

Introduction
Many times, the inert food produced for the feeding of the different culture stages of the aquatic commercial species does not guarantee the organisms obtain the best growth response or well-being conditions [1]. Even if a similar diet was formulated like organisms consumed in natural form, the substitution of live food, especially in the stage of fish fry and crustacean larvae is important so that this production activity should be important to the successful development of aquatic organisms' cultures [2]. Based on the works of Gándara *et al.*, [3], Negrete *et al.*, [4], Castro *et al.*, [5], and Rojas [6] among the most important organisms used as live food found are cladocerans, mainly those species of the genus *Daphnia* sp. that due to their short life cycle, easy culture system, high population growth rates and the ability to modify their nutritional values, is an organism with very high productive potential and that allows its application in these early stages of the life cycle of fish and crustaceans.
The genus *Daphnia* sp. is composed of about 100 different species, commonly known as water fleas. This group comprises planktonic crustaceans that inhabit freshwater inland aquatic systems with a high concentration of organic matter particles suspended in the water like bacteria, yeasts, and microalgae, whose characteristics determine the nutritional quality of these organisms [7]. It is because of this that the production of *Daphnia* sp. under laboratory conditions is a great alternative, because environmental variations can be controlled, and the quality of the food can be managed using different diet sources Ocampo *et al.*, [8] have observed better results in the use of green, red, and brown microalgae for similar purposes to those presented in the present research.
Considering the value and use of cladocerans in aquaculture, the following study aims to compare the population density of the cladoceran *Daphnia* spp. in an experimental culture fed with *Scenedesmus* sp. and *Pinnularia* sp. diets with the addition of yeast, because it is an important source of proteins and carbohydrates that complements their nutrition [9] and thus, find the best treatment for their production in laboratory conditions and be used as live food for the first stages of fish and crustaceans with commercial importance, as well as aquatic ornamental fishes.

Materials and Methods

Obtaining of organisms

Daphnia sp. organisms were obtained taking a 20 L water sampling at Centro de Investigaciones Biológicas de Cuemanco (CIBAC) and taken to Live Food Production and Biofloc Laboratory from Universidad Autónoma Metropolitana Unidad Xochimilco, México.

Experimental design

Plastic containers of 200 L capacity were used and filled with 140 L of tap water at 25 °C temperature with constant air and light. Each container was inoculated with 500 organisms and fed with three experimental treatment diets: treatment 1) *Pinnularia* sp (3 L) + *Scenedesmus* sp (3 L) + Yeast (100 mL); 2) treatment 2: *Pinnularia* sp (6 L) + Yeast (100 mL); and 3) treatment 3: *Scenedesmus* sp (6 L) + Yeast (100 mL). Every third day 1 L sampling from each treatment was taken to obtain 10 subsamples of 5 mL and all cladoceran stages were counted.

Data analysis

All data were introduced in a database in Excel 2021 to obtain the descriptive analysis. All data were extrapolated to 140 L culture volume to obtain the population density growth curves for each experimental treatment.

The population density values from each treatment were placed in a Life Table program to obtain the following reproductive parameters:

Reproduction rate: $Ro = \sum l_x m_x$

Where:

∑= summary

lx= survival proportion from each sampling phase

mx= organisms produced for each live organism from each sampling phase

$$\text{Instantaneous growth rate (r)} = \frac{\text{Log}_e R_o}{T_c}$$

Where:

$\log_e R_o$= natural logarithm of reproduction rate

T_c= generational time of cohort

Generational time of cohort

$$Tc = \frac{\sum x * lx * mx}{R_o}$$

Where:

∑= summary

lx= survival proportion from each sampling phase

mx= produced organisms from each sampling phase

Ro= reproduction rate

Results

The mean values of *Daphnia* sp. population density from each experimental treatment during 60 culture days are shown in Table 1.

Table 1: Mean values (±S.D.) of population density of *Daphnia* sp. from each experimental treatment.

Sampling days	*Pinnularia*+ *Scenedesmus* +Yeast	*Pinnularia*+ Yeast	*Scenedesmus*+ Yeast
0	165±27	134±10	110±14
3	252±34	202±11	167±21
6	385±35	304±29	253±32
9	589±37	458±32	384±35
12	902±37	689±39	583±41
15	1380±53	1037±43	884±61
18	2112±58	1560±51	1341±62
21	3232±60	2347±56	2033±65
24	4945±64	3532±79	3083±106
27	7567±69	5315±87	4675±116
30	11579±95	7997±94	7090±131
33	17718±97	12033±100	10752±140
36	27111±107	18106±109	16306±144
39	41486±110	27245±117	24728±148
42	63482±118	40996±142	37499±150
45	97140±130	61687±148	56867±166
48	148644±139	92820±163	86239±169
51	227456±147	139668±166	130781±171
54	348053±164	210159±167	198328±175
57	532592±165	316229±178	300763±179
60	814973±172	475832±178	456105±180

The organisms fed with both microalgae and yeast showed the highest density with 814,973 org 140 L^{-1}. The other experimental treatments obtained density values of 456,105 org (*Scenedesmus* +yeast) and 475,832 org (*Pinnularia* +yeast).

The growth density curves of *Daphnia* spp. and their respective formula from each experimental treatment are shown in Fig.1.

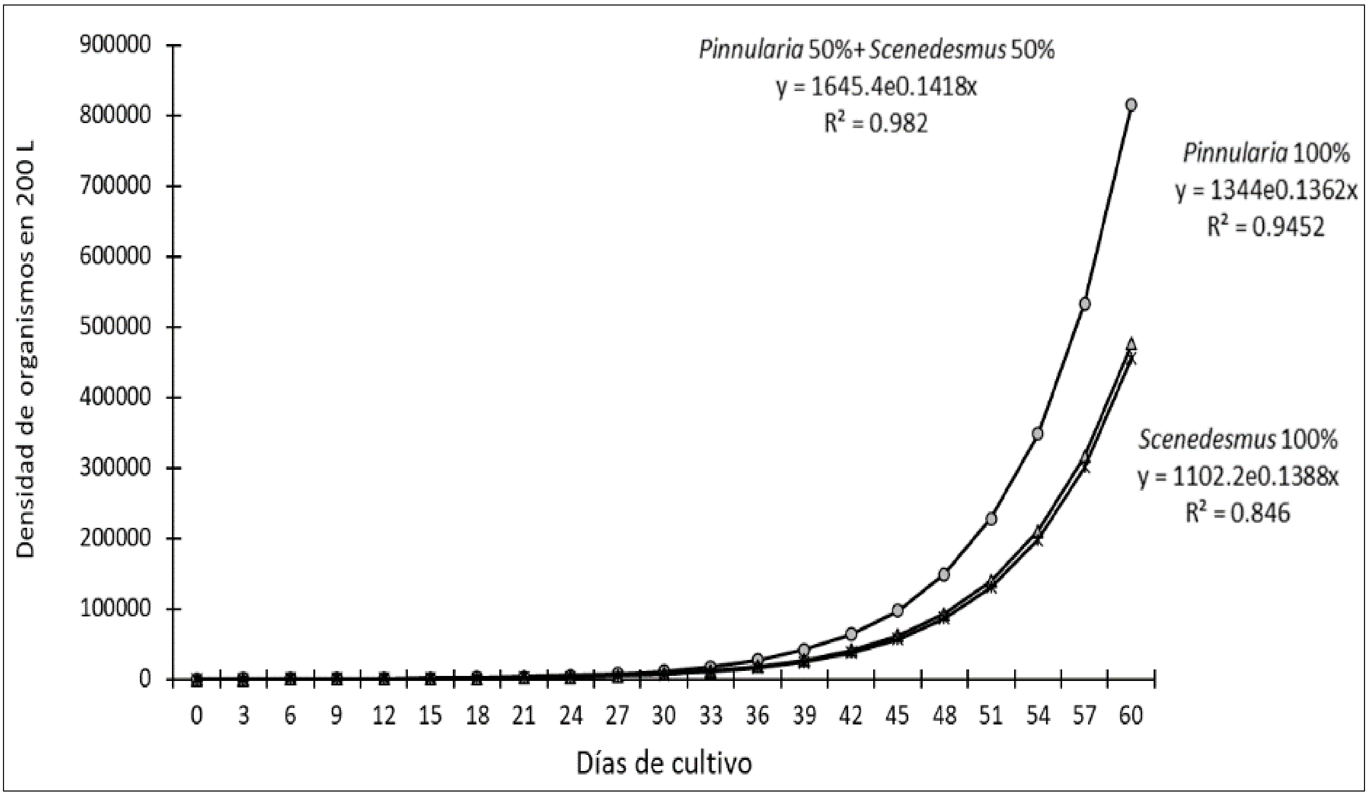

Fig 1: Growth density curves of *Daphnia* spp. Population from each experimental treatment

The reproductive values are shown in Table 2.

Table 2: Reproductive values of *Daphnia* sp. cultured from each experimental treatment.

Experimental treatments	Organisms produced per female (Ro)	Cohort generational time (Tc)	Growth intrinsic rate (r)
Pinnularia + *Scenedesmus* + Yeast	4, 953	17.12	0.50
Pinnularia + Yeast	3, 539	17.02	0.48
Scenedesmus + Yeast	4, 137	17.07	0.48

The highest values of reproductive potential of *Daphnia* spp. cultured at each experimental treatment (Ro, Tc, and r) were shown at mixed diet (*Pinnularia* + *Scenedesmus* + Yeast), the lowest values were shown with *Pinnularia* + Yeast diet.

Discussion

Regarding the management of mixed microalgae diets, Castro *et al.*, [10] reported favorable results when they used chlorophytes and diatoms in *Daphnia pulicaria* cultures, especially when they were used in mixed form. These authors found that if they use a proportion of 25% chlorophytes and 75% diatoms allows densities of up to 2,614,758 org in 140 liters of culture, considering that this *D. pulicaria* has a small size and can have higher densities in the same liters of culture than this experiment, where the density of organisms reached was only 814,973 org in the same 140 liters. According to studies by Ashfaq *et al.*, [11] microalgae show high contents of proteins, lipids, and carbohydrates. Sharma *et al.*, [12] mention that diatoms, such as *Pinnularia* sp. have a high nutritional value that can be used as a food source.

Pérez *et al.*, [13] worked with *Ceriodaphnia cornuta* fed with three different microalgae: *Scenedesmus obliquus*, *S. capricornutum* y *Nannochloris oculata* at different temperatures (20, 25 y 30 °C) obtained high reproductive rate values with *S. obliquus* (r=0.13) and *S. capricornutum* (r=0.25) at 25 °C. In this experiment, at 25 °C temperature culture system obtained the highest values with *Scenedesmus* sp with r=0.48 for *Scenedesmus* + yeast and r=0.50 for treatment with *Pinnularia* + *Scenedesmus* + yeast. The difference was obtained for the yeast supplementation in this research which supports a good source of carbohydrates, lipids, and vitamins. Ocampo *et al.*, [8] who worked with *Daphnia magna* made the same conclusion with this work because they also used yeast at different concentrations and obtained better growth results. They concluded that *D. magna* shows better adaptation to culture medium because yeast supports an adequate lipid concentration and vitamin B_{12}, to promote not only growth, reproduction, and well-being condition to this crustacean.

Since 2019, cultivation of this crustacean began not only with microalgae but also with a carbohydrate source that allows the production of biomass. One of this research is Sadi *et al.*, [14], which added every seven days 0.5 g of molasses at 60 L container with *Daphnia* sp. with an initial density of 100 org. They obtained production in the first 20 days. At the beginning, it had a density of 2 org L^{-1}, while at the end obtained 6,500 org L^{-1}, which gives a production of 390,000 organisms. If we extrapolate the value to 140 L, we obtain 910,000 organisms, a value above the highest production of this experiment with *Pinnularia* + *Scenedesmus* + Yeast, which was 814,973 org 140 L^{-1}. A difference of 95,027 organisms.

Taipale *et al.*, [15], who used microalgae for the restoration of water body quality also obtained densities of *Daphnia* sp.

mention that food quality is the main factor influencing the population dynamics that occur in natural systems, but it is the same that happens in smaller containers because they can contribute to determining the type and concentration of essential fatty acids as well as sterols contained in the different sources of microalgae used for this purpose [16, 17, 18]. They also mention that cladocerans cannot convert eicosapentaenoic acid from 18:3w3 chain polyunsaturated fatty acids in an efficient way so they have to be incorporated into the diet and a main source of it are brown microalgae, as in this case study *Pinnularia* sp Taipale *et al*., [15] mentions that the genus *Daphnia* sp. reproduces more easily in the presence of green microalgae cryptophytes, chrysophytes, and in some cases in brown algae [19] and that when exposed to this mixture along with an external carbohydrate source such as molasses can reach larger sizes than when in a natural environment. This improvement in reproduction and growth in this crustacean is due to the high content of amino acids, w3, and w6 chain fatty acids, as well as sterols concentration in microalgae or carbohydrate source [15].

Bezerra *et al*., [20] worked with *Daphnia magna* using the microalga *Scenedesmus acuminatus* at a concentration of 1.5 x 107 cell mL^{-1} compared to the use of cattle manure (6.5 gL^{-1}) and a combined diet of both during 21 days of culture, finding that the organisms reproduced better in the mixed diet with *S. acuminatus* and cattle manure. These authors mention that the production of this crustacean is related to the quantity and quality of the food supply, as well as its growth and locomotor capacity, which is very important as it is a filter-feeding organism. These authors point out that the use of a single diet with microalgae can compromise the reproductive process not only of *D. magna*, but also of many other cladoceran species. Jasni *et al*., [21], who worked with *Scenedesmus* sp. in dry and paste form, compared with yeast alone, found that when *Scenedesmus* sp is ingested in frozen or dry form the organisms show 75 % fecundity, while when used in paste form a range of 55-95% fecundity is obtained in female cladocerans.

Authors such as Ku *et al*., [22], mention that cladoceran biomass can be measured by the number of organisms or the total weight obtained by the population of this small crustacean. This biomass can be considered as energy that is capable of passing from lower trophic levels to a higher trophic level (down-up), when this live food is available to be consumed by other benthic invertebrates and fish, whether ornamental or the first stages of commercial fish.

Therefore, it is important to carry out these studies in which the diets used in these micro invertebrates meet the nutritional requirements, not only for their growth and reproduction to obtain large amounts of biomass but also for their composition when used as a live food whose nutritional components are the most suitable for the species that consume it.

Conclusion

According to the results obtained in the present work, the individual diets of *Scenedesmus* sp. and *Pinnularia* sp. provide a food source rich in proteins, lipids, and carbohydrates useful for increasing the population density of *Daphnia* sp. However, the combination of these two microalgae in a single diet and enriched with a source of fatty acids and vitamins such as yeast proves to be a supplement of higher nutritional value and, therefore, is an appropriate food that influences the reproductive rate, longevity and reproductive potential of cladocerans under laboratory conditions.

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