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Density population comparison of *Daphnia pulex* Müller, 1785 cultured in laboratory conditions, fed with three green unicellular microalgae (*Sphaerocystis* sp., *Chlorella vulgaris* and *Haematococcus pluvialis*).

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ABSTRACT

This laboratory experiment was focused to determine density population of *D. pulex* fed with three microalgaes: *C. vulgaris, H. pluvialis* and *Sphaerocystis* sp. (600 mL each one at 5 x 10^6 cells mL^{-1} concentration). A fourth diet was a combination from each microalgae in equal parts (200 mL each one). In addition, every third day, 2 mL of yeast (100 g in 4 L of water) was added to culture medium. For daphnias culture medium, 20 L plastic beakers with 10 L of water (three per diet) were used. The temperature was maintained at 25 ± 2°C, a pH of 7-8, and continuous light and aeration. The initial density was 2,000 organisms per beaker. Every third day, a 500 mL sample was taken and from that sample, 10 aliquots of 5 mL were taken to obtain density average (± S.D.). With obtained data, an organism's growth tendency curve from each experimental diet was determined. Growth formula was a sixth grade polynomial. Results show that after 12 days of culture, density increase in all diets, being *H. pluvialis* diet the one that reached the highest density (19,331 ± 60 org 10 L^{-1}) and *C. vulgaris* diet showed the lowest density values (9,762 ± 89 org 10 L^{-1}). Although *C. vulgaris* and combined diets did not show high values, density production maintained constantly during all culture period. Used microalgaes in this experiment provided relevant information about massive production of this crustacean, allowing to obtain and scale culture systems to produce enough densities of *D. pulex* destined to feed aquatic species used in commercial and research activities.

Key words: *Daphnia pulex*, green microalgae, density, growth tendency curves.

INTRODUCTION

Live food in aquaculture plays an important role in the optimal development of aquatic organisms, because many times, it has observed that inert diets like flakes or pellets, were easily reject by cultured animals (Romero-Gamboa 2002). Also, it can be easily produced; its distribution is better in all culture beakers without deteriorating culture medium; has better quality nutritional content; can founded in different sizes (different stages); has better digestibility therefore better cleanliness in culture medium because this live food do not dissolve in water as conventional food and do not reach decompose polluting it (Luna 2013). The live food supply to aquatic organisms gives better results at early stages in fishes and crustaceans (fry and larvae), which are the most problematic stages before passing through live food to inert diet conditions, causing higher levels at survival, development and sexual maturity in culture organisms (Ocampo et al. 2010).

It is common that zooplankton species such as ciliates: paramecium and rotifers; insects: mealworms larva; crustaceans: copepods, ostracods and *Artemia*, are used as live food in aquaculture for their easy breed and the possibility to changes or modified their nutritional values, either because they are filter feeders or consumed food type (Castro et al. 2003). Among crustaceans, two freshwater genus have great relevance in aquaculture industry: *Daphnia* sp.

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and *Moina* sp. The genus *Daphnia* sp. consists 100 species approximately, the best-known are: *D. magna*, *D. pulex*, *D. longispina*. This genus consist in planktonic crustaceans, that feed with suspended particles in water (filter feeders) (Ocampo et al. 2010). *D. pulex* develops at temperatures of 27-28°C and do not survive at extreme temperature changes. These organisms live in habitats where O_2 concentration is variable because they may grow at full saturation levels or even values below 2 mg L^{-1}. High densities of "water fleas" are found in habitats with high organic matter concentration, where bacteria, yeast and microalgae proliferate, from which this crustacean feeds (Torrentera and Tacon 1989). "Water flies" supports very low ranges of heavy metals and industrial waste, which kill them easily (Martinez et al. 2010).

Under laboratory conditions, *D. Pulex* production may be relatively easy,, because environmental conditions can be controlled due to the possibility of supply different diets that can be use as food. However, the best results were observed when unicellular green microalgae preferably *C. vulgaris* are being used. In this study, the use of *C. vulgaris* and other two microalgaes (*H. pluvialis* and *Sphaerocystis* sp.) additional to a combined diet of the three microalgaes, will provide more information about microalgal diets used in these crustaceans culture technique and can be applied in another research or massive laboratory production to obtain better mass production densities.

MATERIAL AND METHODS

Obtention of organisms. *D. pulex* organisms used in this experiment were obtained from San Gregorio Atlapulco, Xochimilco locality (Fig.1). The collected organisms were place in a 200 L plastic beaker with 160 L freshwater and fed with unicellular green microalgae during one week for acclimatization.

Microalgae culture. Microalgae were cultured in 20 L plastic beakers filled with 19 L of freshwater, previously filtered, de-ionized and de-chlorinated. They were fertilize with 10 mL of

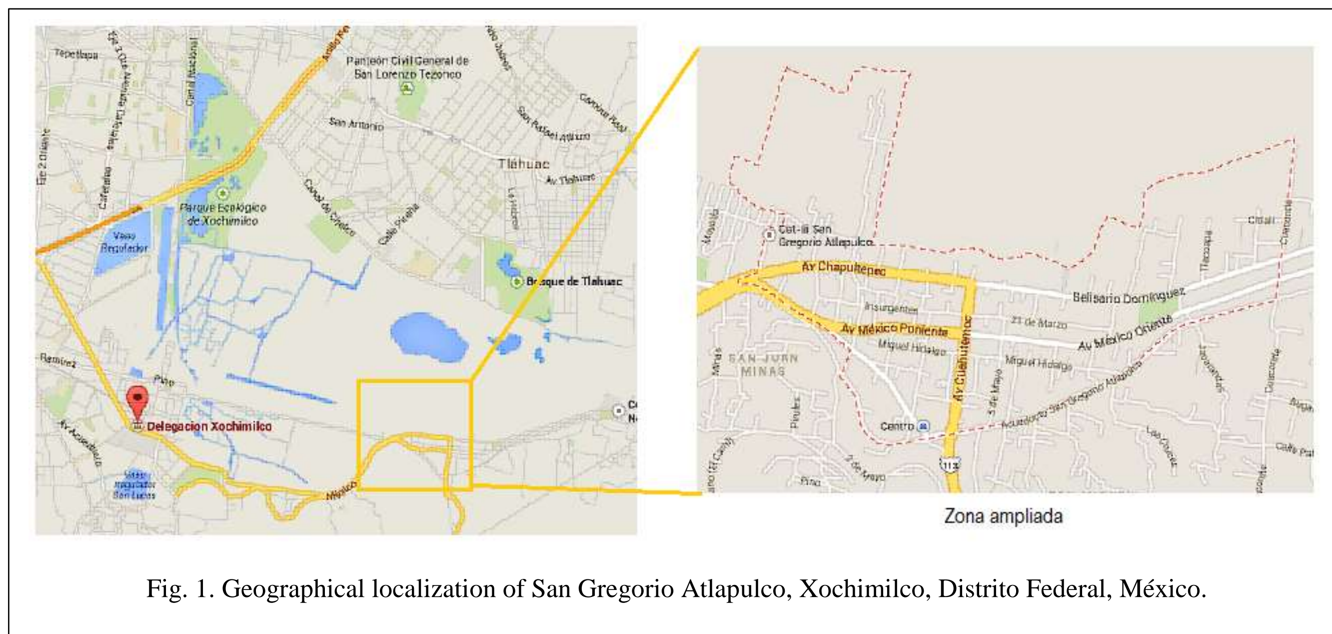

Fig. 1. Geographical localization of San Gregorio Atlapulco, Xochimilco, Distrito Federal, México.

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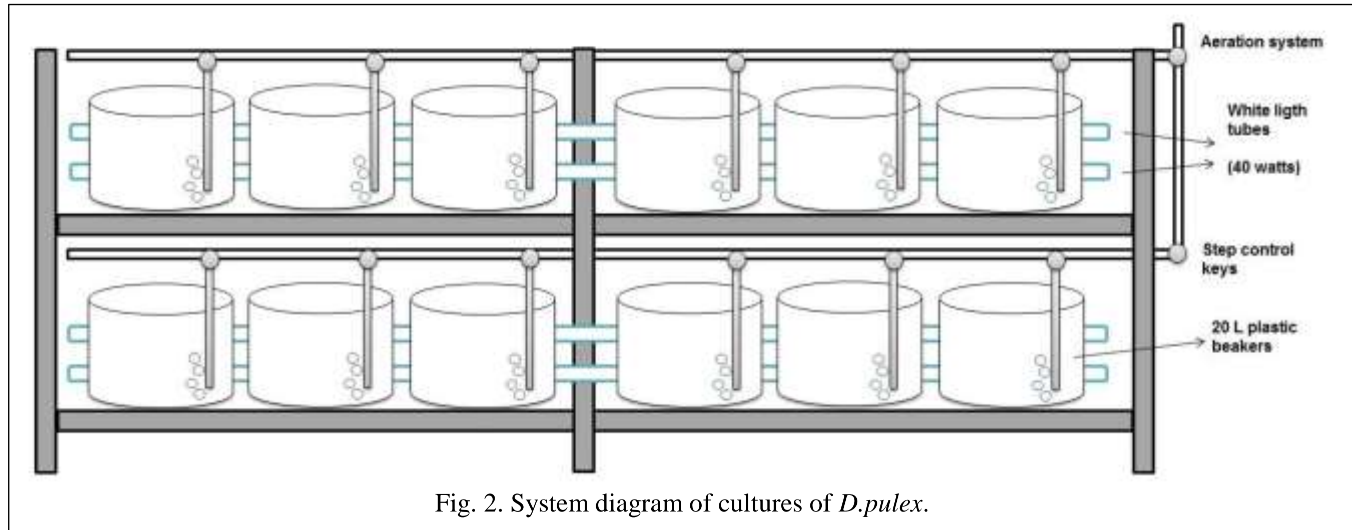

Fig. 2. System diagram of cultures of *D.pulex*.

Triple 17 (500 g 500 mL^{-1} of water) and 5 mL of Urea (1 Kg 4 L^{-1} water). Each week, 50% of the microalgae medium was changed to avoid the microalgae concentration of 5 x 10^6 cells mL^{-1}.

Experimental design. 20 L plastic beakers (three for each diet) were installed. Temperature at 25 ± 2°C, a pH between 7-8 units, and continuous light and aeration were maintained (Fig.2). The acclimated population of *D. pulex* was concentrated in six liters of water from wich 500 mL were taken for inoculation each beaker of 20 L capacity with 10 L of water. In each beaker, 600 mL of microalgae was supply: 1) *C. vulgaris*; 2) *H. pluvialis*; 3) *Sphaerocystis* sp. and 4) combined diet (all three microalgaes in equal parts, 200 mL). Every third day, 2 mL of dry active yeast was added (100 g 4 L^{-1} of water) as dietary supplement.

Sampling and data processing. Every third day, *D. pulex* were counted to determine population density. For that it were taken 500 mL of sample from each beaker and from this sample 10 aliquots of 5 mL were counted to determine mean values (± S.D.). A database was elaborate in Excel 2010 program to obtain descriptive statistics for diet and sampling data. The data were statistically processed using Systat 13 program, to determine significant differences ($P<0.05$) using a one-way analysis of variance (ANOVA). Also through Excel 2010 program, tendency growth curves of population density were obtained.

RESULTS

Table 1 show mean values (± SD) of *D. pulex* density population fed with four experimental diets per culture day. Highest densities are shown with *H. pluvialis* diet with density values of 19,000 org 10 L^{-1}. *Sphaerocystis* sp. and combined diets reached densities of 12,000 to 13,000 org 10 L^{-1}, while lowest density is observed with *C. vulgaris* diet with a 9,000 to 11,000 org 10L^{-1} range.

The ANOVA tests show significant differences ($P<0.001$) between sampling days from each diet and between experimental diets. Only in *C. vulgaris* diet no significant differences were found between first and sixth sample day and; between 18 and 30 days of sample ($P =1.000$) (Fig. 3).

Fig. 4, shows the growth tendency curves of *D. pulex* density population fed with four experimental diets. All the formula diets showed a polynomial curve of sixth grade.

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Table 1. Mean values (±D.S.) of *D. pulex* density per experimental diets per sample days.

Culture days	Experimental diets			
	Sphaerocystis sp.	*Chlorella vulgaris*	*H. pluvialis*	Combined
0	2,000	2,000	2,000	2,000
	±25	±25	±41	±55
3	3,329	1,673	1,820	1,918
	±22	±22	±42	±54
6	2,755	2,018	1,640	1,835
	±31	±44	±44	±42
9	2,213	3,201	4,609	4,030
	±48	±22	±50	±46
12	6,688	6,141	7,272	2,409
	±73	±23	±56	±53
15	11,786	8,821	9,756	8,099
	±82	±98	±46	±36
18	12,490	9,742	12,676	13,070
	±80	±99	±69	±30
21	9,139	9,377	16,163	11,837
	±68	±101	±49	±39
24	7,298	9,640	19,211	8,903
	±42	±97	±48	±38
27	11,538	11,358	19,331	11,943
	±37	±142	±60	±50
30	13,095	9,762	12,517	10,733
	±24	±89	±69	±58

DISCUSSION

In *D. pulex* laboratory cultures have been used different live foods, inert diets or a combination of them, obtaining different information about density populations. This experiment did not study which microalgae diet was better with respect others, only shown results that allow aquaculture producers take decisions about what microalgae should can be use, as well as incorporation of inert food (dry active yeast) or make two or three microalgae combinations to obtain massive productions of *D. pulex*.

As mentioned above, there are studies with these cladocerans (*D. pulex*) which have different results. Rojas et al. (1999), worked with *D. pulex* in a 14 L culture medium at 22°C using three diets with wheat bran: a) wheat (10 mL), b) wheat bran + radish juice) and c) wheat bran + spinach juice. Both in 1:50 relation, supplied twice a week. Initial culture density was 200 org 14 L^{-1}, the culture period was 21 days. Density results per diet were 1,722; 7,997 and 8,921 org 14 L^{-1} respectively. Density increased 400-500% when vegetable juices (inert diet) were supplied. Even so, these values are lower than those obtained in this experiment with microalgae in 5 x 10^6 cells mL^{-1} in 10 L concentration, which density increases above 600-700%.

Alva-Martinez et al. (2001) worked with *D. pulex* and *Moina macrocopa* species, fed with *C. vulgaris* at three different concentrations (0.75, 1.5 and 3.0 x 10^6 cells mL^{-1}); these authors obtained density values of 3, 4 and 6 org mL^{-1} respectively. These density values are higher than those founded in this research with *C. vulgaris*, with a difference of 28,642 org 10 L^{-1} with the same microalgae diet. The same increase in density was observed with *M. macrocopa* culture, where the average density reaches 50 x10^3 org 10L^{-1}, also fed with *C. vulgaris* diet. Mangas-Ramirez et al. (2001) obtained densities of *D. pulex* of 2 org mL^{-1} fed with *C. vulgaris* at 1.5 x 10^6 cells mL^{-1} concentration; while in this experiment density reached 1 org mL^{-1} with the same microalgae diet. Densities of 2 org mL^{-1} were found with *H. pluvialis* diet.

Sanchez-Ortiz et al. (2010), worked with *Ceriodaphnia dubia* and *D. pulex* fed with *Scenedesmus acutus* obtaining densities of 2 org mL^{-1} in *D. pulex*, similar to what was found with *H. pluvialis* diet in this experiment. These authors mentioned that densities values depend on culture medium temperature, food concentration and daphnias body size. When the first two conditions

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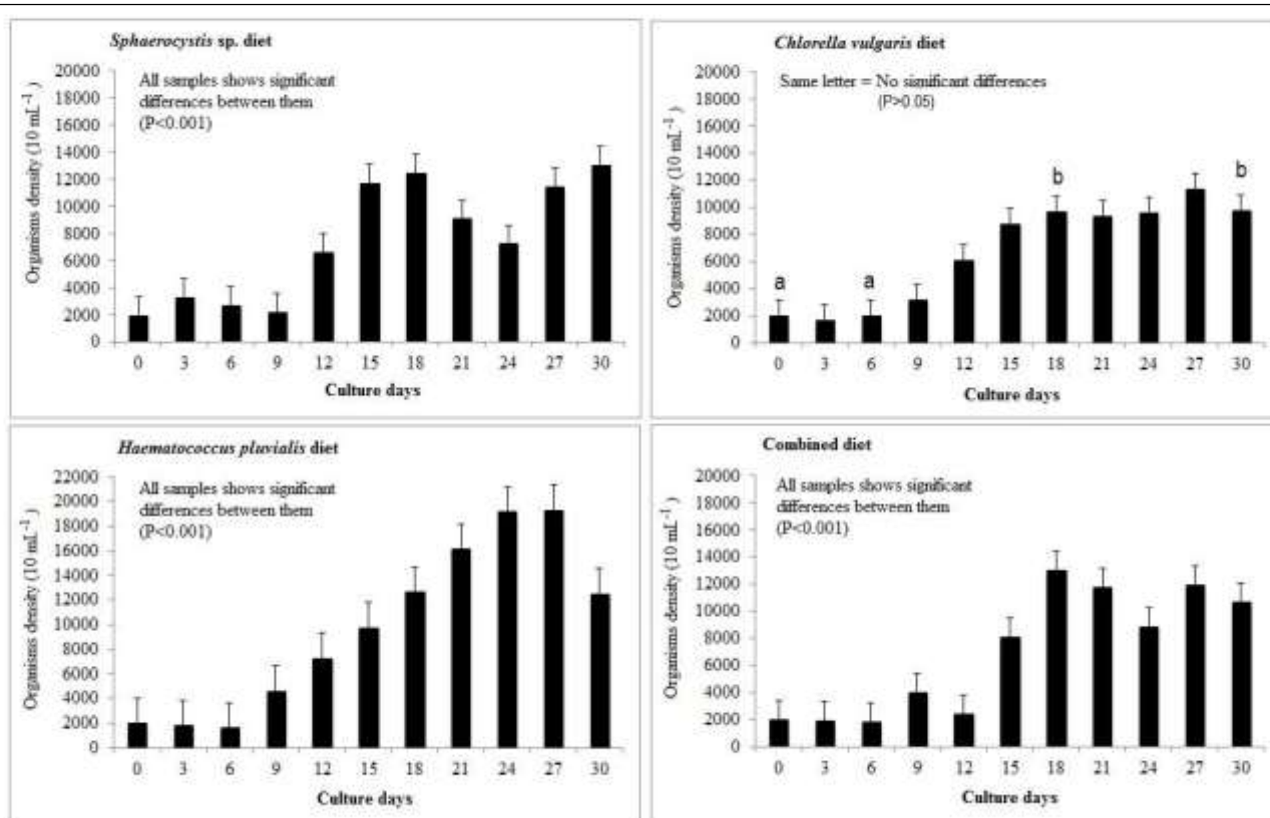

Fig. 3. ***Daphnia pulex*** **density population variance analysis between samples from each experimental diet.**

were properly controlled, the density variation depends only on the body size, being larger species those who get lower culture densities than smaller species. Sanchez-Ortiz et al. (2010), which studied with *C. dubia* obtained 5 org mL^{-1} densities, three more organisms per milliliter than those obtained in *D. pulex*. Nandini and Sarma (2003) mentioned that cultures with bigger cladocerans, microalgae concentrations must decrease in culture medium, because organisms have low rates production than those cladocerans cultures with small sizes organisms. These small organisms can reach sexual maturity quickly and contribute to increase the crop density (Alva-Martinez et al. 2007). Feniova et al. (2013) reported the same conditions with *D. pulex* (considered a large cladoceran with a length of 1.6 ± 0.06 mm). These authors also mentioned that population density decrease quickly at 27°C culture medium than being at 20°C.

Gama-Flores et al. (2011) worked with *D. pulex* and *C. dubia* using *C. vulgaris* as food (0.01, 0.1 and 1.0 x 10^6 cells mL^{-1} concentrations) and three different temperatures (15, 20 and 25°C). *D. pulex* had a better density at 1.0 x 10^6 cells mL^{-1} of microalgae and a temperature of 20°C in culture medium with values of 50 org 40mL^{-1}. This value was bigger than those values founded in this study with *C. vulgaris* diet, but 50% less than values founded with *H. pluvialis* (80 org 40 mL^{-1}). These authors mentioned that population density do not change only with microalgae concentration supply but also response to specific temperature culture medium.

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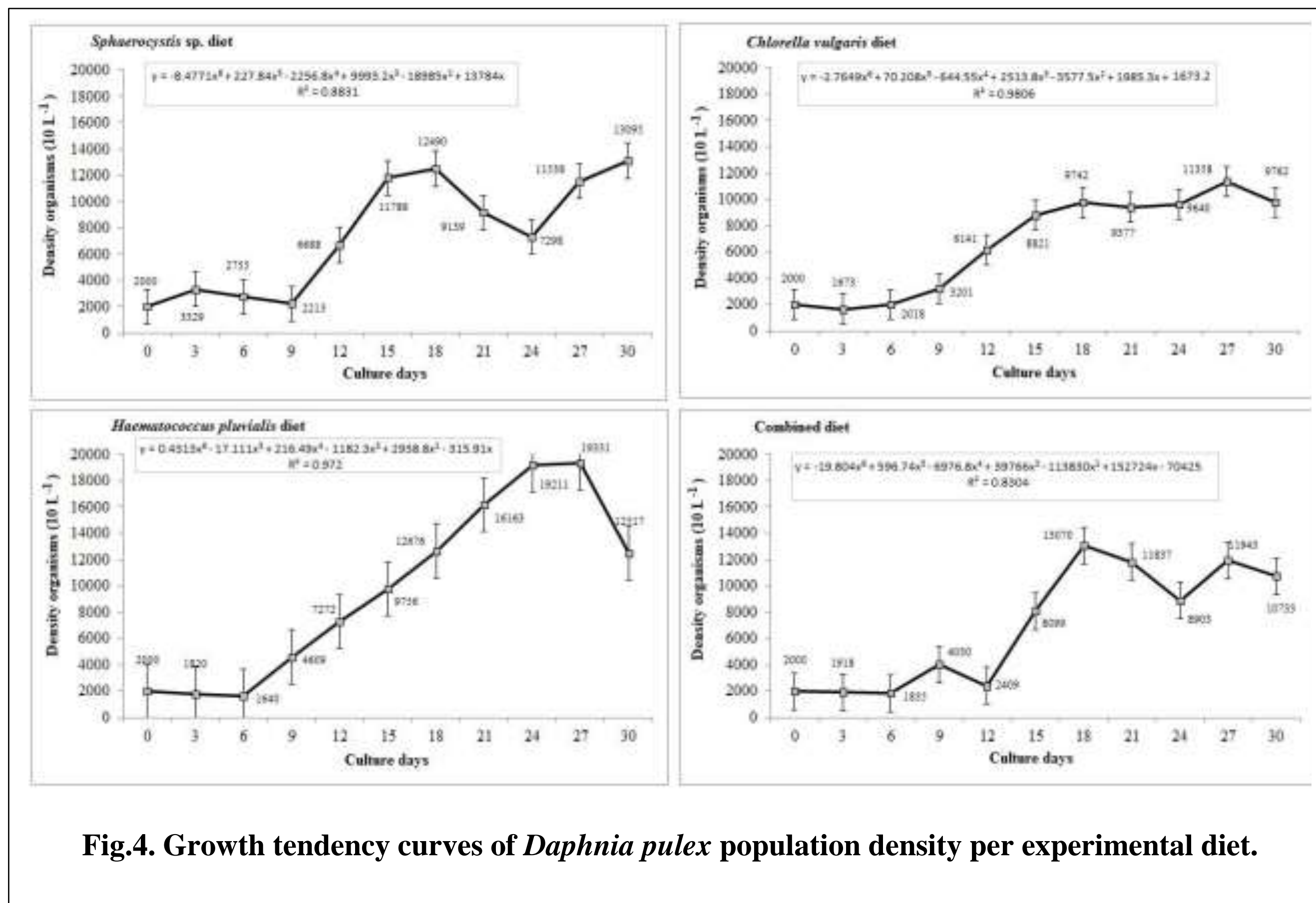

Fig.4. Growth tendency curves of *Daphnia pulex* population density per experimental diet.

This is why these two variables need to be monitored constantly in cladocerans culture medium.

Usually an increase of food concentration (microalgae) in *D. pulex* culture medium does not assure a culture success. This increase in food concentration may cause a loss in fecundity in females, which has an intraspecific competition for space (Kilham et al. 1997; De Mott et al 1998; Jong et al. 2014). Munoz and Martinez (2007) suggest that is important to consider the number of adequate females at the beginning of the culture to assure best opportunity of fertility rates and consequently increase density in culture beakers and not to wait until female are produce and reach the sexual maturity and began to produce new organisms.

Is important to consider the microalgae cell wall composition in cladocerans culture, because digestive capacity of some cladocerans could decrease and consequently a low nutrient absorption could increase fertility and regeneration time as mentioned by Van Donk and Kilham (1990).

Finally, Jong et al. (2004) mentioned that in cladocerans cultures it is important to use diatom microalgae to improve digestive capacity of *D. pulex*. Another good characteristic is their better nutritional content in lipids and carbohydrates than green microalgaes. This better nutritional quality increase the female reproduction rates, decrease time females reach sexual maturity and begin reproduce faster and consequently, generate new and high population density in culture beakers.

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CONCLUSIONS

The *H. pluvialis* diet show higher density values (50% up) with respect other experimental diets (2 org mL^{-1}); all other diets reached 1 org mL^{-1} density values.

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