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Laboratory production of *Daphnia magna* (Straus 1820) fed with microalgae and active dry yeast

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Abstract

Daphnia magna cultivation was made using four experimental diets: 1) *Chlorella* sp., 2) *Haematococcus* sp. 3) *Sphaerocystis* sp. and 4) a combination of previous microalgae, at a concentration of 500×10^3 cell mL^{-1} and as supplement active dry yeast. Feeding and population count took place every third day. Highest population density per liter was found in *Chlorella* sp and combined diet (3,693 and 3,600 org L^{-1}). And the lowest density was found in *Sphaerocystis* sp. diet with 2,900 org L^{-1} . Life tables showed a production of 41 and 43 organisms per female in this diets while *Haematococcus* sp. and *Sphaerocystis* sp. diets with 35 org per female. Highest growth rate presented in *Chlorella* sp. diet (0.82) and the lowest in combined diet (0.52). Although, it presents a better stability in organism quantity during the whole experiment. The obtained results allow this cladocerans producer's take better used diet decisions.

Keywords: Cladocerans, density, microalgae, diets, life tables, tendency curves

1. Introduction

Fish nutrition is very important in aquaculture production, since it affect survival and growth of cultured species, determining the commercial success of this industry [1]; this is particularly true in early larval stages when mortality is high because offspring have high nutritional requirements for growth and development, but still not well developed digestive enzyme activity that allows them to assimilate nutrients inert food. Also it has been observed that commercial diets are not specific and do not contain required nutrients for all cultured species [2]. In this context, live food production is emerging as a strategy to improve cultivated species nutrition. Live food is a natural source of proteins, lipids, carbohydrates, minerals and vitamins; additionally, does not modified water quality and its color, smell and permanent movements, stimulate predator to consume [3].

Crustacean *Artemia franciscana* and rotifer *Brachionus* sp. are some of the organisms that are more used as live food [4]. Nevertheless, new species have been used as live food, like copepods and cladocerans. From these species, *Monia* sp. and *Daphnia* sp. gender are the most used in aquaculture [5] due to their high nutritional value, high culture densities, adequate size, soft body, short life cycle and mobility.

Daphnia sp. is a group of filtering organisms that mainly consume microalgae [6], but they also use bacteria and detritus so their culture is easier. Nevertheless, it is required to delve in productive aspects as their growth and population density under different culture conditions and feeding, to establish its relevance in fish larvae feeding. So the aim of this study is to evaluate the density of *Daphnia magna* fed with three microalgae supplemented with active dry yeast under laboratory conditions, not only to maintain the population to laboratory studies, also to produce live biomass in semi intensive conditions or dry biomass to use it as raw material to produce inert diets to fed fishes or crustaceans.

2. Material and Methods

Experiment was run for 80 days period, considered from the collection in natural environment, isolation of individuals and population growth used for experimental design that lasted 60 days.

2.1 Sampling

D. magna organisms were obtained from a water sample of 20 L from culture ponds belonging to Centro de Investigaciones Biológicas y Acuícolas de Cuernavaca (CIBAC), of Universidad Autónoma Metropolitana Unidad Xochimilco (UAM-X). The sample was sieved (100 µm mesh) and observed under a light microscope (Olympus SZ40) to separate specimens.

2.2 Increase population density

Once cladocerans were separated, they were maintained in 20 L container, with continuous light and aeration conditions. Organisms were fed every third day with 0.5 L of each one

green microalgae: *Chlorella vulgaris*, *Haematococcus fluviatilis* and *Sphaerocystis* sp. at 500 x 10³ cell mL⁻¹ concentration and 10 mL of active dry yeast (200 g 4 L⁻¹ concentration) to increase population density.

2.3 Experimental design

From 20 L container, 100 cladocerans were taken and placed in 4 L plastic containers. Experiments were made by triplicate in each experimental diet. Cultures were kept at 25±2°C temperature, pH 7-8, and continuous light and aeration supply during 60 days. Every third day 100 mL of each bottle are taken to measure population density (Fig.1).



Fig 1: Culture system used for *D. magna*.

2.4 Food supply

Four experimental diets with green microalgae were used: 1) *C. vulgaris*; 2) *H. fluviatilis*; 3) *Sphaerocystis* sp. and 4) Combination of three microalgae. All of them at 500 x 10³ cell mL⁻¹ concentration. Also 0.5 mL of active dry yeast (200 g 4 L⁻¹ of water) was added to diets each third day.

2.5 Information process

Obtained density data every third day, was registered in an Excel 2010 spreadsheet to obtain the average value and its standard deviation (±S.D.). The tendency growth curve was determined for each experimental diet, as the life tables considering that:

Reproduction rate (Ro):

$$R_o = \sum l_x m_x$$

Where:

l_x= Survival proportion from each phase.

m_x= Organisms produced from each phase / Organisms observed from each phase

Cohort generation time (Tc):

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

Where:

x = Phase

∑x l_x m_x= Produced organisms sum by each original individual in each phase.

Ro: Reproduction rate

Instantaneous growth rate (r):

$$r = \log_e R_o / T_c$$

Where:

Log_eR_o= Base e logarithm of reproduction rate.

T_c= Cohort generation time.

Survival proportion in each phase (l_x):

$$l_x = a_{x+1} / a_{inicial}$$

Where:

a_(x+1)= Organisms quantity in previous phase.

a_{inicial}= Organisms quantity at beginning of experiment.

Life expectancy (e_x):

$$e_x = T_x / l_x$$

Where:

T_x= time left to live.

l_x= Survival proportion in each phase.

3. Results

Organism densities mean values (±S.D.) from each experimental diet are shown in Table 1. The production peaks were: *C. vulgaris* 3,663 ±191 org 1,000 mL⁻¹ at 33 culture day; *H. fluviatilis* 2,900 ±192 org 1,000 mL⁻¹ at 30 culture day; *Sphaerocystis* sp. 2,500 ±149 org 1,000 mL⁻¹ at 39 culture day; combined diet 3,600 ±183 org 1000 mL⁻¹ at 45 culture day. Meanwhile, population mortality in each experimental diet occurred at: 42 culture day to *C. vulgaris*, 57 culture day to *H. fluviatilis* and *Sphaerocystis* sp. and at 51 culture day to combined diet. Maintaining high densities per 1000 mL⁻¹ in each different diet was as follows: *C. vulgaris* with 3,000 org (15-33 culture day); *H. fluviatilis* with 2,000 org (24-54 culture day); *Sphaerocystis* sp. with 2,000-3,000 org (21-42 culture day) and combined diet with 2,000-3,000 org (21-48 culture day).

In Fig. 2 tendency growth curves of *D. magna* population density are shown. All curves were adjusted to grade three polynomial growth curve.

Table 1: Average value (±S.D.) of *D. magna* population density in 1000 mL of sample.

Muestreo	<i>C. vulgaris</i>	<i>H. fluviatilis</i>	<i>Sphaerocystis</i> sp.	Combinada
0	100	100	100	100
	±18	±16	±10	±17
3	165	300	195	240

	±18	±20	±10	±16
6	270	630	225	240
	±19	±25	±13	±20
9	308	795	135	150
	±20	±15	±9	±12
12	438	750	435	240
	±24	±15	±16	±11
15	3,631	720	360	285
	194	16	±13	±22
18	3,153	990	1,200	600
	±181	±13	±148	±15
21	3,139	1,395	3,165	2,370
	±191	±155	±157	±161
24	3,140	2,350	2,440	3,270
	±200	±148	±147	±182
27	3,050	2,220	2,350	2,700
	±206	±165	±161	±165
30	3,650	2,900	2,250	2,700
	±183	±162	±145	±176
33	3,663	2,650	2,346	3,150
	±191	±148	±140	±160
36	2,600	2,800	2,498	3,450
	±188	±159	±152	±164
39	1,960	2,500	2,500	3,300
	±193	±158	±149	±160
42	0	2,700	2,450	3,300
		±163	±157	±159
45		2,800	1,950	3,600
		±164	±161	±183
48		2,750	1,650	3,300
		±158	152	±167
51		2,700	1,050	0
		±162	±146	
54		2,550	300	
		±156	±19	
57		0	0	

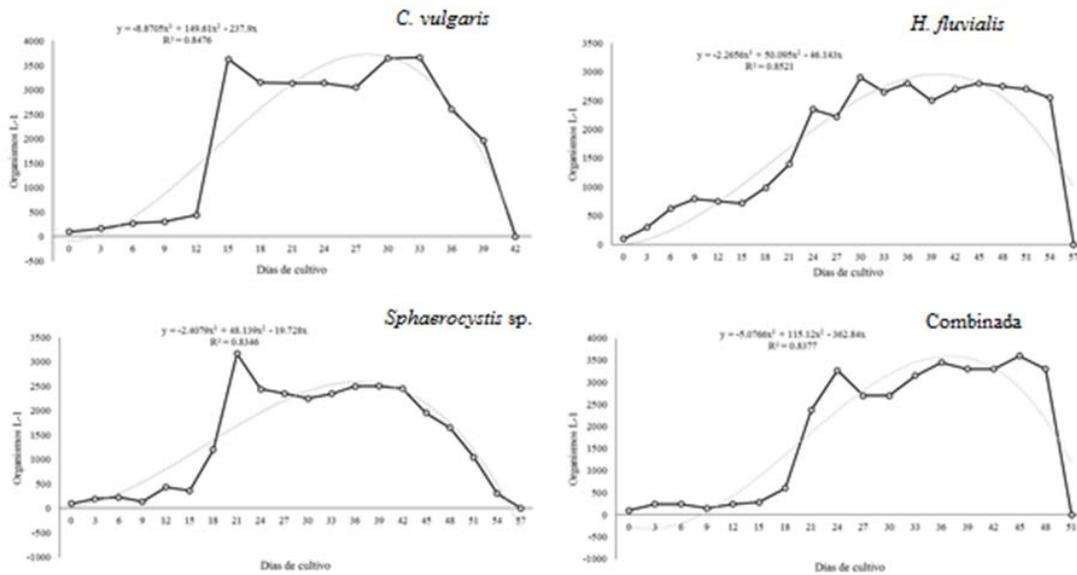


Fig 2: Tendency growth curves of *D. magna* population density cultured with experimental diets.

Life table values of each experimental diets are shown in Table 2. It can be seen that *C. vulgaris* and combined diets had a higher production with 41 and 43 org per female respectively; while both *H. fluviatilis* and *Sphaerocystis* sp. diets had 35 org per female. Concerning growth rates, the highest occurs with *C. vulgaris* diet with a 0.82 value and the lowest occurs with combined diet with 0.52 value.

Table 2: Life Tables of *D. magna* population fed with the four experimental diets: a) *C. vulgaris*; b) *H. fluvialis*; c) *Sphaerocystis* sp. and; d) Combined microalgae.

a) <i>Chlorella</i> sp. diet							b) <i>Haematococcus</i> sp. diet								
Culture day	Organisms density (k ₀)	Survival proportion (k ₁)	Organisms produced per female	Reproduction rate (R ₀)	Cohort generation time (T _c)	Expectancy life (-ex)	Culture day	Organisms density	Survival proportion (k ₁)	Organisms produced per female	Reproduction rate (R ₀)	Cohort generation time (T _c)	Growth rate (r)	Expectancy life (-ex)	
0	100	1.00	65	41	4.56	0.32	292.16	0	100	1.00	300	3.5	6.61	0.53	345.50
3	165	1.65	105				176.26	3	300	3.00	330				114.50
6	270	2.70	38				106.91	6	630	6.30	655				53.79
9	308	3.04	130				92.78	9	795	7.95	0				41.73
12	438	4.34	3103				64.30	12	750	7.50	0				43.20
15	3631	3.631	0				7.21	15	720	7.20	270				43.93
18	3133	3.133	0				7.23	18	990	9.90	405				31.12
21	3139	3.139	2				6.26	21	1395	13.95	955				21.23
24	3140	3.140	0				5.23	24	2330	23.30	0				11.81
27	3030	3.030	660				4.30	27	2700	27.00	680				11.47
30	3650	3.650	13				2.75	30	2900	29.00	0				7.90
33	3653	3.653	0				1.74	33	2650	26.50	150				7.59
36	2690	2.690	0				1.25	36	2800	28.00	0				6.21
39	1990	1.990	0				0.20	39	1500	15.00	200				5.90
42								42	2700	27.00	100				4.50
45								45	2800	28.00	0				3.36
48								48	2750	27.50	0				2.41
51								51	2700	27.00	0				1.44
54								54	2330	23.30	0				0.26

c) <i>Sphaerocystis</i> sp. diet							d) Combined diet								
Culture day	Organisms density (k ₀)	Survival proportion (k ₁)	Organisms produced per female	Reproduction rate (R ₀)	Cohort generation time (T _c)	Expectancy life (-ex)	Culture day	Organisms density	Survival proportion (k ₁)	Organisms produced per female	Reproduction rate (R ₀)	Cohort generation time (T _c)	Growth rate (r)	Expectancy life (-ex)	
0	100	1.00	85	35	5.63	0.53	275.49	0	100	1.00	240	43	7.18	0.52	329.45
3	195	1.95	30				140.52	3	240	2.40	0				136.56
6	225	2.25	0				120.85	6	240	2.40	0				135.56
9	135	1.35	390				200.09	9	150	1.50	90				215.60
12	425	4.25	0				61.44	12	240	2.40	45				133.54
15	360	3.60	840				73.14	15	285	2.85	315				111.87
18	1200	12.00	1965				21.29	18	600	6.00	1770				52.49
21	3155	3.155	0				7.28	21	2370	23.70	900				12.64
24	2440	2.440	0				8.43	24	2270	22.70	0				8.30
27	2330	2.330	0				7.73	27	2700	27.00	0				8.04
30	2230	2.230	96				7.05	30	2700	27.00	450				7.94
33	2346	2.346	152				5.78	33	3150	31.50	300				5.88
36	2498	2.498	2				4.46	36	3450	34.50	0				4.41
39	2590	2.590	0				3.46	39	3300	33.00	0				3.39
42	2430	2.430	0				2.22	42	3300	33.00	300				2.50
45	1930	1.930	0				2.04	45	3600	36.00	0				1.42
48	1630	1.630	0				1.22	48	3300	33.00	0				0.50
51	1030	1.030	0				0.79	51							
54	300	3.00	0				0.20	54							

5. Discussion

In laboratory *D. magna* cultures have employed a variety of living, inert or combination of both diets, with different density organism's results. In this experiment we sought to provide the necessary information for farmers which requiring produce this live food, take a decision to use a single or combined microalgae diet or if used microalgae and yeast combinations.

With respect physical and chemical cladocerans culture conditions, optimal temperature range was 20-28 °C; pH 7-8 to ensure adequate development and metabolism of organisms [7]. Must be careful with microalgae supply because it can decrease pH concentration culture medium and affect organism's growth [7], so it is recommended addition of 210-260 mg L⁻¹ of calcium carbonate (CaCO₃) [8]. In this study, the physical and chemical conditions and microalgae density were remained constant during all experimental period.

It is important began a cladocerans culture with 150 org L⁻¹ [12]. In this study the initial culture density was 100 org L⁻¹, however, final densities were considered good. The optimal densities vary depending cladocerans species and their size, because bigger ones requires more space like *D. magna*. *Moina* sp. needs 12.3 ± 0.3 org mL⁻¹ density to begin their cultures using both microalgae and yeast diet, which can stimulate high population growth [9], four times above obtained with *C. vulgaris* diet (3.6 ± 1.91 org mL⁻¹) in this experiment. Some authors mentioned that used different diets as alfalfa juice (low-cost diets) obtained high population densities (302 org 2 L⁻¹) [23], values below this experiments (7,200 org 2 L⁻¹).

Bigger cladocerans did not need high food concentration in their culture medium, because they have low growth rates, regarding smaller ones [15]. Smaller cladocerans reached faster sexual mature than bigger ones and therefore contribute to increasing culture density [16-21]. High microalgae concentration at culture medium can decrease female

fecundity induced by space competition [24-26]. Similarly, microalgae cell wall composition can reduce digestive capacity and slow nutrient absorption, decreasing fertility and population regeneration [28]. It has recommended to use microalgae diatom's to improve digestive capacity and provide better nutritional content in terms of lipids and carbohydrates, unlike green microalgae. This nutritional intake stimulates growth, reaching sexual maturity and reproduction rate in less time [26-27]. It is important to highlight in cladocerans production culture, use of diatom microalgae as complement food diet, because this microalgae can improve digestive capacity, increase their lipid and carbohydrates content, which increase reproduction rates and faster growth to reach sexual maturity and begin to reproduce to regenerate population density [26].

Food concentration must be reduced when cladocerans size was bigger [15], because they have low production rate, regarding the smaller cladocerans ones, which production increases faster because they reach sexual maturity faster than bigger cladocerans and therefore contributes to culture density [16-21]. High cladocerans population growth rates, can be associated to their parthenogenetic type of reproduction and their high neonate's production. Other author's points out that generally cladocerans culture experiments go through pronounced growth cycles [22]. In cladocerans dry yeast diets supply, rich with Vitamin E, stimulate population growth. *Moina* sp. cultures shows growth rate of 0.36 and cohort generation time of 1.94 days [9], in *D. magna* experiment, shows higher growth rate (0.82) and lowest cohort generation time (4.56 days) with *C. vulgaris* diet.

Photoperiod in culture medium is an important variable to be considered [10]. Continuous periods of light and darkness affect their behavior and impact directly in their locomotion, feeding, reproduction and molt patterns. An increase of microalgae density can obstruct digestive tract and in worst case, produce a toxin that eliminate. This is not presented in

D. magna experiments, because remained at continuous light conditions, organisms density remained 2,000 and 3,000 org L⁻¹, unlike *Moina* sp. cultures with a 12 hours of light continuous which obtained only 349.2 ± 75 org L⁻¹ [10]. Some authors [13-14] mentioned that at a lower quantity of light produced more male ratio stage and therefore decrease in lower quantity of reproductive events in cladocerans populations.

D. magna can reach growth rates of 0.182 with a photoperiod of 12 hours of light, a well below value with what was found in this experiment, because obtained growth rates were of 0.52-0.82 and cultures were kept with continuous light during all day [11]. *D. magna* cultures with an initial density of 25 org L⁻¹, using yeast as food enriched with highly unsaturated fatty acids, obtained densities of 826 org L⁻¹, with a growth rate of 0.258 and a cohort generation time of 2.69 days [6]. Those values were below with this experiments data, because densities reached 2,500-3,663 org L⁻¹ and higher growth rate 0.52 to 0.82.

Finally, it should be noted that information generated in this experiment allows a proper use and handling of cladocerans, especially in *D. magna*, in this work case, facilitating the choice of food to be used: microalgae, yeast, or mixtures, to generate cultures according to the needs for aquatic organisms consumption or experimental purposes: increased production, fast or slow growing cultures, to determine harvest period to maintained constant density cladocerans populations. The producer has the final decision.

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