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Population density comparison of *Ceriodaphnia dubia* fed with bacteria obtained from Biofloc system

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Abstract
The use of heterotrophic bacteria as the food source in cladocerans diet to make massive production cultures has been little studied. So, this experimental study used *Ceriodaphnia dubia* cultivated in triplicate in 20 L plastic beakers at 19°, 21° and 25°C temperature, for 60 days. The bacteria source was obtained from screened liquid (20 µm) grown in Biofloc tilapias system. Every third day a sample of 500 mL was taken from each beaker and all organisms were counted. The maximum density was obtained at 19°±2°C was of 130,560±136 org, whereas, lower density was found at 25°±2°C with 107,833±461 org. The ANOVA analysis showed significant differences (P<0.001) between experimental treatments. Obtained reproduction rates values were r=0.058-0.063; Ro=12-14 org. per female and Tc=42 days. However, heterotrophic bacteria can be used as food to maintain cladocerans low density culture or in mixed diets with microalgae.

Keywords: *Ceriodaphnia dubia*, cladocerans, bacteria, culture, temperature, reproduction rates

1. Introduction
The zooplanktonic species like rotifers (*Brachionus calyciflorus* and *B. rubens*), insects, cladocerans (*Moina macrocopa* and *Ceriodaphnia dubia*) and *Artemia* are used as live food in aquaculture industry for their facility to be cultured and capacity to change their nutritional value [1], using different microalgae and inert diets for evaluate zooplankton population growth patterns [2, 3].
One of these zooplankton populations was the cladocerans called "water flies", because their little size and characteristic jump movement in water [4]. These organisms belong to the freshwater family Daphniidae, which was cosmopolite, with short life cycle. Depending temperature culture conditions, they can live for 13 to 60 days range [5]. Water quality, nutritional value and food quantity variables, affect their reproduction rate and frequency, and their population growth in culture medium [6]. Also, cladocerans show different responses and sensibility to diverse diets, low content of nutrients in microalgae or inert diets [7, 8], which affects directly organism's development. A technique that has not been employed to feed cladocerans was the use of heterotrophic bacteria produced in Biofloc system, which were rich in vitamins and mineral sources, especially phosphorus [9].
Therefore, the goal of the study was to consider heterotrophic bacteria produced in the tilapia Biofloc system like food, to produce massive cladocerans culture (*Ceriodaphnia dubia*) in 20 L plastic beakers in the laboratory, considering their use when microalgae culture falls or they do not have optimal cell concentration.

Material and methods
Organisms supply
Ceriodaphnia dubia strain was obtained from cladocerans stock of the Live Food Production Laboratory from Universidad Autónoma Metropolitana, Xochimilco unit.

Experimental design
Nine plastic beakers (20 L) filled with 15 L of freshwater were used to make culture experiments. Light (40 w, white tube) and aeration were constant (Fig.1). They were tested at three experimental temperatures: 19°, 23° and 25°C for triplicate.

The organisms were fed with heterotrophic bacteria produced in the tilapia Biofloc system. Every third day, organisms were sampled and counted to determine population density for 60 days.

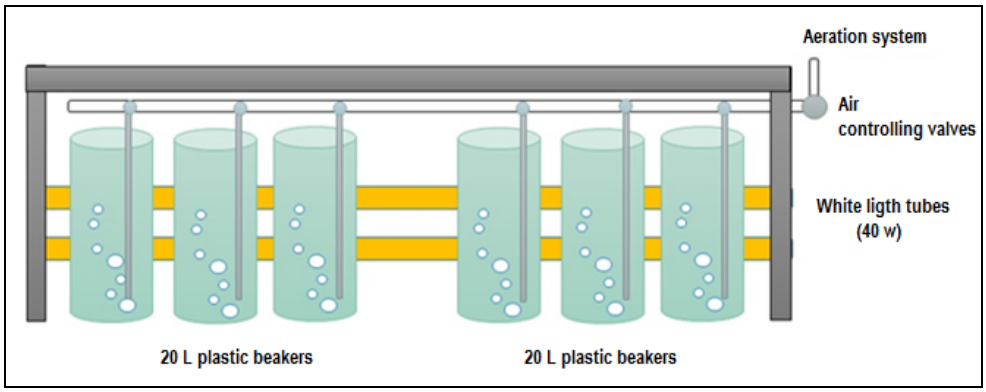

Fig 1: Experimental design of *Ceriodaphnia dubia* at three experimental temperatures (19°, 23° and 25 °C) fed with heterotrophic bacteria produced in the tilapia Biofloc system.

Bacteria production (Biofloc)

Three weeks before initiating *Ceriodaphnia dubia* experiments at different temperatures, Biofloc system was installed in two plastic beakers of 200 L capacity, filled with freshwater at 160 L, with vigorous aeration and temperature of 25°C. Juvenile stage tilapia organisms (35) were introduced and fed with extruded pellets (5%) with 60% of protein content and enriched with molasses (3%) as the carbohydrates source of total tilapia population weight.

Cladocerans feeding

Previously, every day, 500 mL of cladocerans culture medium was extracted and sieve through a 10 µm mesh to keep organisms. Then, 5 L of Biofloc production beakers, was extracted and sieve through a 10 µm mesh and 500 mL of this culture medium (rich in bacteria), was applied for each culture cladocerans beaker.

Sampling

Every third day (for 60 days), from each experimental beaker, 500 mL were taken and sieve through 10 µm mesh. The organisms were concentrated in 50 mL and three subsamples of 1 mL were taken, fixed with Lugol solution (5%) and counted using an Stereoscopic Microscope Leica EZ4HD.

Data processing

Population density values were introduced in Excel 2010 data base to obtain mean values (±S.D.) and extrapolated to 20 L culture medium. Also, growth tendency curves were obtained. Density values were introduced in the Life Table Program (Excel 2010) to obtain reproductive parameters:

Reproduction rate: $Ro = \sum l_x * m_x$
Where:
∑= summatory
lx = survival proportion from each phase
mx = produced organisms from each survival organism from each phase

Growth intrinsic rate: $r = \log_e R_o / T_c$
Where:
$\log_e R_o$ = reproduction rate natural logarithm
T_c = Cohort generational time

Cohort generational time: $Tc = \sum x*lx*mx/Ro$
Where:
∑= summatory
lx = survival from each phase
mx = produced organisms from each phase
Ro = Reproduction rate

Statistical analysis

Significant differences ($P<0.05$) between cladocerans experimental culture medium at different temperatures were determined by ANOVA analysis. When this analysis shows significant differences, a multiple mean values comparison (Tukey's test) was made using Systat 13.0 statistical program.

Results

Table 1 shows the mean values of population density every third culture day. The experimental cultures at temperature conditions of 19°±2 °C obtained highest density with 130 560±136 org.20 L^{-1}, whereas at 25°±2 °C showed the lowest density with 107 833±461 org.20 L^{-1}. At 60 days of culture, ANOVA analysis showed significant differences ($P<0.001$) between experimental temperatures.

Table 1: Mean values (±S.D.) of population density of *Ceriodaphnia dubia* cultured at three experimental temperatures.

Sampling	Experimental culture temperatures		
	19°C	23°C	25°C
0	8 974±97	8 782±79	8 897±61
3	5 270±14	5 250±15	5 471±12
6	2 595±17	2 671±73	2 926±10
9	951±16	1 044±60	1 263±61
12	336±67	369±76	480±13
15	751±86	646±68	580±14
18	2 196±31	1 876±81	1 560±38
21	4 671±72	4 057±64	3 422±24
24	8 175±100	7 191±105	6 166±110
27	12 710±140	11 277±160	9 790±164
30	18 274±198	16 316±189	14 296±199
33	24 869±225	22 306±529	19 684±295
36	32 493±320	29 249±723	25 953±207
39	41 147±402	37 144±204	33 103±224
42	50 830±484	45 991±448	41 135±848
45	61 544±547	55 790±758	50 048±475
48	73 288±672	66 542±623	59 842±237
51	86 061±727	78 245±272	70 158±764
54	99 864±898	90 901±598	82 075±985
57	114 697±100	104 509±192	94 513±219
60	130 560±136	119 070±364	107 833±461

*Ceriodaphnia dubia g*rowth tendency curves (Fig. 2) showed better results at 19°C. All curves were polynomial grade two and R^2 values were up 0.90 correlation.

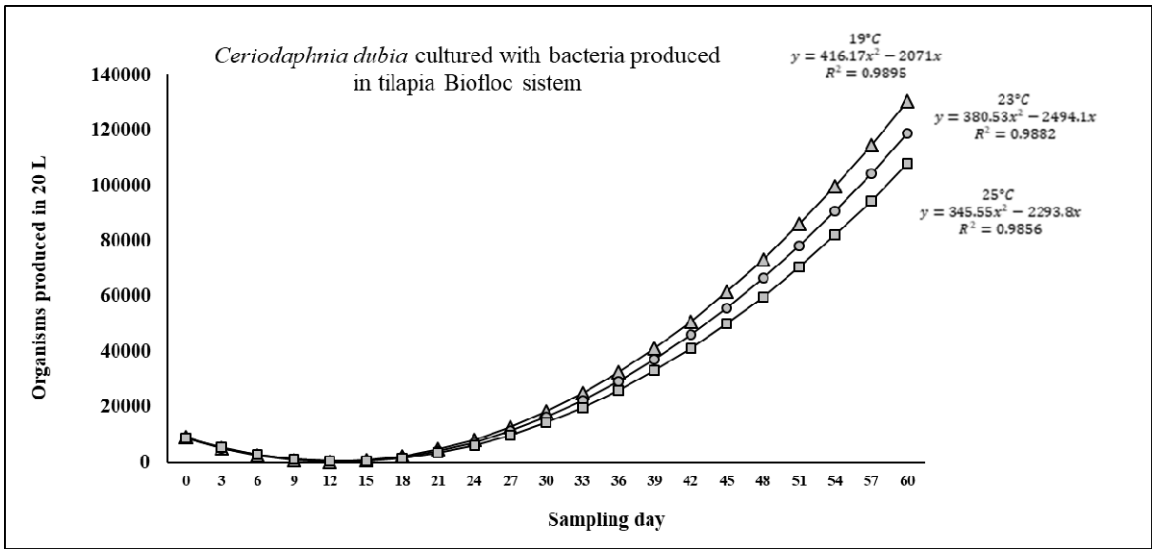

Fig 2: Population density tendency growth curves of *Ceriodaphnia dubia* produced in laboratory at three experimental temperatures and fed with bacteria produced in the tilapia Biofloc system.

Table 2 shows the Life Table values. Organisms produced per female (Ro) changed according to temperature condition, obtaining for 19 °C experimental test 14 organisms; for 23°C, 13 organisms and for 25°C only 14 organisms. The reproductive variables (Tc and r) did not show differences.

Table 2: Production values of *Ceriodaphnia dubia* produced in laboratory at three experimental temperatures.

	Reproduction rate $\sum lx*mx$ **Ro**	**Cohort reproduction time** $\sum x*lx*mx/Ro$ **Tc**	**Instantaneous growth rate** $\log_e Ro/Tc$ **r**
19 °C	14,5108	42,567	0,063
23 °C	13,5169	42,682	0,061
25 °C	12,0656	42,874	0,058

Discussion

For a massive cladocerans culture, it is important to maintain constant carbon (C) and phosphorus (P) levels [10], since in diets with deficiency of phosphorous cause morphological changes in some cladocerans populations. This condition can be maintained with a mix diet of microalgae and bacteria. Bacteria present in cladocerans culture medium or applied externally like complement diet [10] can be used as food, because as well as providing essential fatty acids (EFA), it provides essential amino acids and vitamins. Another source of EFA was the diatom microalgae, which can be applied in cladocerans culture medium and not only green microalgae as monoculture, which is rich in proteins and pigments. This combination of bacteria+green microalgae+ diatom microalgae can improve cladocerans culture [11].

It is important to considered culture food density, because low values, can reduce feeding rates and decreasing population growth and reproduction [12], but in high cladocerans culture densities can provoke that invested energy in motherhood, changes life cycle strategies in next cladocerans generations, producing bigger females that grow and mature more quickly [12].

Cladocerans organisms showed adaptive plasticity at different oxygen concentrations (O_2) and temperature wide ranges [13]. These environmental conditions were regulated by organism's genetic pool, in which different proteins, to maintain adequately cellular function were acting. That's why important to apply a diet source, not only with good digestion, but also with high protein content. The temperature affects principally biochemical reaction in organism's physiology, which can be modified cladocerans fecundity and reproduction, also can affects heartbeats, respiration process and muscular activity. For this reason, it is important to consider that organisms need to take an acclimation period to obtain better survival rates [13]. In this study, *C. dubia* had an acclimation time (21 days) before being tested in experimental temperature, which allowed population grow until 60 cultured days.

Essential amino acids are important too, because not only are used for maintenance and well-being of cladocerans [14], but also the deficiency of Arginine: Histidine or Lysine: Threonine induce ephippia formation in *Daphnia pulex* populations [14].

With respect to growth rates in cladocerans populations, it's mentioned that not all cladocerans species or populations respond equally to different environmental conditions and food sources, especially in Life Table values [15]. These authors mentioned that in low food concentration, females produce higher size eggs with a lot of yolk to resist food low concentration and began diminished organism production. Poor nutritional diet cause that cladocerans modify their reproduction rates, interchanging growth, and reproduction type at the population level. This was apparently observed when in cladocerans culture microalgae diet was suppressed [16].

Although bacteria cannot be considered as a high nutritional

diet for zooplankton, it can be ingested and settle in their digestive tract [17], but when bacteria are defecated, and go into culture medium, they are exposed to environmental conditions and only few can be favored to reproduce in culture medium and used again as food for zooplankton. Bacteria presence in culture medium for their ingestion in cladocerans must be sourced by decomposition of dead organisms or their exoskeleton covers [18]. These organic matter sources were important to bacteria culture present in cladocerans culture medium, because it can be a nutrient regeneration source [19].

Also, cannot forget genetic component between cladocerans species or their different populations [20], which allow more efficiency organism's response to selective pressure for space, food, and temperature differences.

Bacteria diet source play a significant role in zooplankton food, but it *C. dubia* case, only can maintained in low growth rates until you can get new microalgae culture or applied like supplement diet rich in vitamins and minerals or some enzymes that bacteria produce.

Conclusion

Heterotrophic bacteria obtained with the tilapia Biofloc system can be used as maintaining food source in microalgae absences or low production. These heterotrophic bacteria can be used as complement diet or as mixed diet with one or two microalgae sources.

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