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Density comparison of *Moina macrocopa* (Straus, 1820) cultured at different temperature conditions (19, 23 and 25 °C) fed with bacteria obtained from Biofloc system

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Abstract
The main goal of this study was to know if heterotrophic bacteria obtained from Biofloc Tilapia system can be used as food source to cladocerans organism. This study was made with *Moina macrocopa*, which was cultured by triplicate in 20 L plastic beakers at 19°, 23° and 25 °C temperature, for 60 days at Live Food Production Laboratory of Universidad Autónoma Metropolitan Xochimilco, during May to June of 2017. The bacteria source was obtained from culture medium of Biofloc system with tilapia organisms, which was screened through 20 µm PVC sieve. Every third day a sample of 500 mL was taken from each culture medium and all organisms were counted. The maximum density was obtained at 23°±1°C with 85,552±255 org, whereas, lower density was found at 19°±1°C with 9,921±219 org. The ANOVA analysis showed significant differences (P<0.001) between experimental treatments. Obtained reproduction rates values were r=0.73 to 0.139; Ro=22 to 192 org. per female and Tc=39.31 to 42.23 days. Tendency curves show a polynomic second grade curves with R^2 up to 0.90 of correlation value. Heterotrophic bacteria can be used as food to maintain cladocerans low-density culture or in mixed diets with microalgae.

Keywords: *Moina macrocopa*, cladocerans, life table values, heterotrophic bacteria

1. Introduction
Different authors mention that cladocerans are an important food source as support aquaculture culture medium, because they are live food, with filtration capacity to be enriched with different nutritional components, and they are an easily catch prey for fish and crustaceans, since first to adult live stages [1].
Cladocerans culture technique was relativity simple, but specific production and fed technique knowledge in these organisms was incipient [2].
Moina macrocopa can be used as live food in aquaculture industry because of their facility to be cultured using different microalgae and inert diets for evaluate zooplankton population density [3-5]. Depending temperature culture conditions, cladocerans culture can live for 13 to 60 days range [6]. Water quality, nutritional value and food quantity variables, affect their reproduction rate and frequency, and their population growth in culture medium [7]. Also, cladocerans show different responses and sensibility to diverse diets, low content of nutrients in microalgae or inert diets [8, 9], affects directly to organism's development. A technique that has not been employed to feed cladocerans was the use of heterotrophic bacteria produced in Biofloc system, which are rich in vitamins and mineral sources, especially phosphorus [10].
Therefore, the goal of the present study was to consider heterotrophic bacteria produced in the tilapia Biofloc system like food, to produce massive cladocerans culture (*M. macrocopa*) in 20 L plastic beakers in the laboratory, considering their use when microalgae culture falls, or they do not have optimal cell concentration.

2. Material and methods
2.1 Organisms supply: This study was made at Live Food Production Laboratory from Universidad Autónoma Metropolitana Xochimilco, during May to June 2017. *M. macrocopa* strain was obtained from cladocerans stock of this laboratory.

2.2 Experimental design
Nine plastic beakers (20 L) filled with 15 L of freshwater were used to make culture experiments. Light (40 w, white tubes) 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.

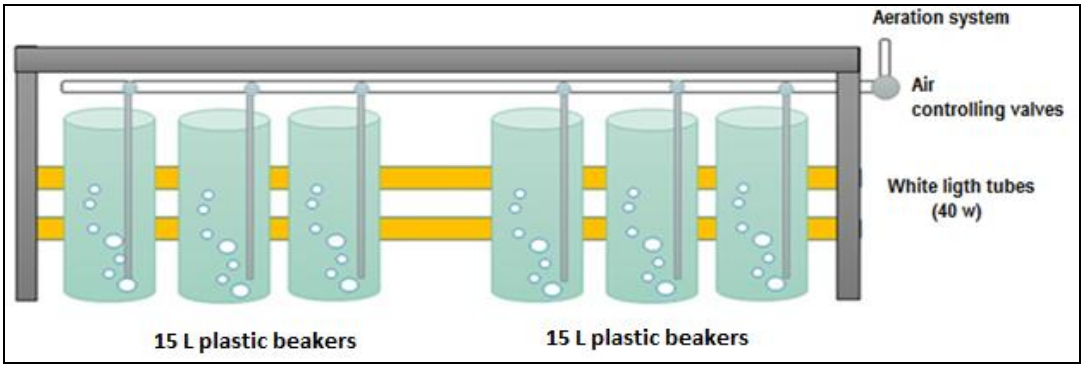

Fig 1: Experimental design of *M. macrocopa* at three experimental temperatures (19°, 23° and 25 °C) fed with heterotrophic bacteria produced in the tilapia Biofloc system.

2.3 Bacteria production (Biofloc): Three weeks before initiating *M. macrocopa* experiments at different temperatures, Biofloc system was installed in two plastic beakers of 200 L capacity, filled with 160 L of freshwater, 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.

2.4 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 beaker of cladocerans.

2.5 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 a Stereoscopic Microscope Leica EZ4HD.

2.6 Data processing: Population density values were introduced in Excel 2010 data base to obtain mean values (±S.D.) and extrapolated to 15 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:
∑= summation
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:
∑= summation
lx = survival from each phase
mx = produced organisms from each phase
Ro = Reproduction rate

2.7 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 12.0 statistical program.

3. Results
Table 1 shows the mean values of population density every third culture day. At temperature conditions of 19 °C it was obtained highest density values of 9921±219 org.15 L^{-1}, at 23 °C of 85552±255 org.15 L^{-1} and for 25 °C showed values of 73439±343 org.15 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 *M. macrocopa* cultured at three experimental temperatures.

Sampling	Experimental culture temperatures		
	19 °C	23 °C	25 °C
0	444±23	444±35	444±21
3	457±61	109±10	551±11
6	285±15	243±9	391±13
9	187±16	1057±13	675±18
12	164±11	2333±29	1403±17
15	215±52	4071±104	2576±176
18	340±40	6271±127	4192±129
21	540±40	8932±132	6252±125
24	815±58	12055±205	8756±175
27	1164±146	15640±156	11704±171
30	1588±187	19687±167	15097±109
33	2086±120	24196±142	18933±133
36	2658±126	29166±192	23213±132
39	3306±106	34598±198	27937±137
42	4027±127	40492±192	33105±133
45	4823±123	46847±147	38718±118
48	5694±194	53665±165	44774±174
51	6639±139	60944±144	51274±274
54	7659±165	68685±168	58218±188
57	8753±357	76888±367	65606±306
60	9921±219	85552±255	73439±343

M. macrocopa growth tendency curves (Fig. 2) showed better results at 23°C. All curves were polynomial grade two and R^2 values were up to 0.90 of correlation.

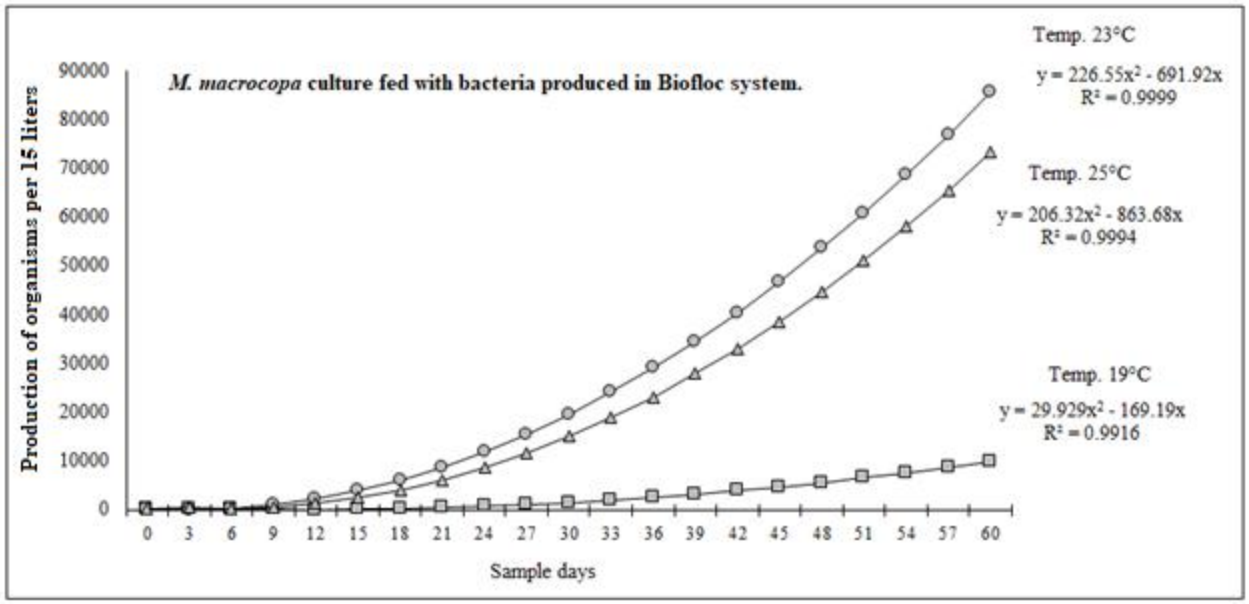

Fig 2: Population density tendency growth curves of *M. macrocopa* produced in laboratory at three experimental temperatures and fed with bacteria produced in the tilapia Biofloc system.

Table 2 shows the Life Table values. Organisms produced per female (Ro) changed according to temperature condition, obtaining for 19 °C experimental test 22 organisms; for 23 °C, 192 organisms and for 25 °C only 164 organisms. The reproductive variables (Tc and r) did not show differences between 23° and 25 °C experimental test, but with 19 °C experimental test showed significant differences (p<0.001) with respect to other temperature test.

Table 2: Production values of *M. macrocopa* produced in laboratory at three experimental temperatures.

Culture temperature	Reproduction rate $\frac{\sum lx*mx}{Ro}$	Cohort reproduction time $\frac{\sum x*lx*mx/Ro}{Tc}$	Instantaneous growth rate $\log_e Ro/Tcr$
19°C	22.00	42.33	0.073
23°C	192.44	39.31	0.139
25°C	164.76	40.27	0.127

4. Discussion

For a massive cladocerans culture, it is important to feed it with a mixed microalga source, but one study mentioned that is important to maintain constant carbon (C) and phosphorus (P) levels [11]. Phosphorous deficiency can cause morphological changes in some cladocerans populations. This condition can be modified if a bacteria source is applied to cladocerans culture system as complement diet [11] like the one obtained through Biofloc system culture with tilapia. Those bacteria source can provide some essential fatty acids (EFA), amino acids, vitamins, and pigments [12]. In *Moina* sp. case, only one study was found using bacterial communities obtained by piggery digestion effluent [13]. The specie cultured was *Moina australiensis* in conical beakers with 50 L of water volume with piggery digestion liquid at 10, 20, 30, 40, 50 and 60 mg L^{-1} concentration. The best "r" values was obtained in 30 mg L^{-1} with 0.15±0.08 and the other concentrations were obtained a 0.02-0.06 "r" range. These results were according with this study, because "r" range was 0.07-0.13. This allow to conclude that bacteria, as only food source, do not allow to get higher cladocerans production, but it can use it, as complementary food. Other study [14], with *Moina australiensis* fed with *Chlorella vulgaris*, which was fertilizer with piggery digestion effluent obtained production of 70-170 org L^{-1}, with and "r" value of 0.09-0.11. Nevertheless, the "r" values were similar with this study, the density values were lower with respect this study (661 to 4,895 org L^{-1}).

Some problems showed in cladocerans culture were those caused by low density food levels like reducing feed rates and decreasing population growth and reproduction [15], but in high density food levels can provoke changes in population life cycle strategies in next generations, producing bigger females that grow and mature more quickly [15].

Cladocerans organisms, in culture systems, showed adaptive plasticity at different temperatures concentrations [16], which were regulated by organism's genetic pool to maintain adequately cellular function at those different temperature conditions. That is why it is important to apply a diet source, not only with good digestibility, but also with high protein content. The temperature affects principally biochemical reaction in organism's physiology, which can modify cladocerans fecundity and reproduction can affects heartbeats, respiration process and muscular activity [17].

Not all cladocerans species or populations respond equally in growth rates to different environmental conditions and food sources, especially in life table values [18]. Some authors [19], which was working with *Moina* sp. cultures fed with *Ankistrodesmus* sp. microalgae and yeast (*Saccharomyces cereviseae*) at 40 x 10^5 cells mL^{-1} concentration, obtained density values of 12.3±0.30 org mL^{-1} and "r" values of 0.36, mentioned that is necessary the yeast source because their vitamin B concentration. These values were higher with respect this study, but confirm that it is necessary to apply mixed diets to obtain better results. Studies made with *Moina* sp. and *Diaphanosoma* sp. fed with *Chlorella* sp. and *S. cerevisiae* [20], obtained values of 5,571±1,037 org L^{-1}, fed with only yeast and 7,936 org L^{-1} with only *Chlorella* sp. source higher values with respect this study with higher

values of 4,895 org L^{-1}. Only yeast or bacterial, used as only food source, not provide the total nutrient concentration that cladocerans populations need, not only to survival, but to obtain higher yield productions.

Another study using liquid waste effluents as fertilizer [21], using human or cow urine and compost (cow/pig) obtained lowers values (468-1,236 org 10 L^{-1}) with respect this study, because with heterotrophic bacteria source can obtained 661 to 4,895 org L^{-1}. "Ro" values were 6-18 org per female and this study obtain 22-164 org per female.

In some cladocerans cultures, low food concentration provoke that females produce higher size eggs with higher yolk concentrations to respond this deficiency and consequently diminished organism reproduction rates or interchanging sexual to parthenogenetic reproduction type. This was observed when microalgae diet was suppressed in cladocerans culture system [22].

Studies shown that bacteria source as food to cladocerans is efficient because it can be ingested and settle in their digestive tract, but it cannot be considered as a high nutritional diet for zooplankton [23]. Bacteria source can reproduce in culture system and can be another protein source to these organisms, but also, bacteria can clean water system eliminating dead organisms or their exoskeleton covers [24]. These organic matter sources are important to bacteria culture present in cladocerans culture medium, because it can be a nutrient regeneration source [25]. Some authors [26], using poultry digestion liquid dropping in three species of zooplankton (*Brachionus calyciflorus, Moina micrura* and *Thermocyclops* sp.) as fertilizer of microalgae, but rich in heterotrophic bacteria, which can be used as nutrient source, obtaining 126±8 org L^{-1} day^{-1} or 8 to 1,291 org L^{-1} range with a "r" value of 0.72-0.92 range and maximum biomass production peak at 6 and 12 culture days. The "r" values were higher with respect this study, but the density organism per liter were lower (661 to 4,895 org L^{-1}).

Also, it cannot be forgetting genetic component between cladocerans species or their different populations [27], 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 in *M. macrocopa* case, it can only be maintained in low growth rates until getting new microalgae culture or applied like supplement diet rich in vitamins and minerals or some enzymes that bacteria produce.

5. Conclusions

Heterotrophic bacteria obtained with the tilapia Biofloc system can be used as maintaining food source in microalgae absences or, if aquaculture or aquarist producer want, to maintain low production culture system.

Heterotrophic bacteria can be used as complement diet (source of some EFA, proteins, vitamins, and pigments) which can supply some nutrient microalgae deficiency.

Using heterotrophic bacteria as only food source cannot supply a suitable nutrient concentration to cladocerans to obtain higher biomass productions.

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