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Comparison of population density of *Simocephalus vetulus* (Müller, 1776), cultured at 19, 23 and 25 °C, fed with bacteria produced in a Biofloc system

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Abstract
The main goal of this study is to test produced bacteria in a Biofloc system to feed, in this case, *Simocephalus vetulus.* This cladoceran was cultured by triplicate in 20 L containers, at three different temperatures (19, 23 and 25°C), during 60 days of culture under laboratory conditions. Bacteria were obtained from the Biofloc culture medium with tilapia, which were filtrated through a mesh of 20 µm. each third day, 10 samples of 10 mL were collected to know the quantity of organisms. The maximum density that was obtained was at the temperature of 23°C with 334,956±437 org $20L^{-1}$. The ANOVA showed significant differences between the temperatures ($P<0.001$). Obtained reproductive values were: r=0.16, Ro=628, and a Tc= 40.92. Growth tendency curves were polynomic of grade two. Produced heterotrophic bacteria in a Biofloc system can be used as food to maintain low densities of culture or as complementary food when microalgae are supplied.

Keywords: *Simocephalus vetulus*, cladocera, life tables, heterotrophic bacteria

1. Introduction
It is known that Cladocera group play an important role in aquatic organisms feeding, mainly in early stages of fish and shrimp commercial species but can be an indispensable food in the maintenance of fresh water ornamental fish, because they are an easy prey for its depredators, due to its slow swim. As a filtering organism, these organisms can be enriched with proteins, carbohydrates, lipids, pigments, probiotics and antibiotics [1].
The main species of Cladocera that have been used as live food are genera *Daphnia* sp. and *Moina* sp. even though there are other genus capable of being used for this purpose as: *Ceriodaphnia* sp. and *Simocephalus* sp.
Cladocera are organisms easy to cultivate because they can use a big variety of unicellular microalgae as food, as well as inert foods like yeast [2-4]. Depending on the nutritional quality of the food, as well as in the temperature of the culture, the cladocera culture can vary 13 to 60 days [5]. It has been observed that the nutritional quality of the food and its quantity, alongside the culture water quality, are variable that can affect the rate and frequency of reproduction and therefore its growth rate in the culture [6]. It also has been observed that different species of cladocera present different answers to the same supplied food (microalgae and yeast), affecting directly the development of the organisms in culture [7, 8]. Even though it has been used yeast for the culture of cladocera, very few has been made with the use of heterotrophic bacteria produced in a Biofloc system.
Biofloc system is described as aggregates (flocs) of algae, bacteria, protozoa and other type of particulate organic matter, as feces and non-consumed food that causes a deterioration of water quality. Each floc is joined together in a loose matrix of mucus that is secreted by bacteria, united by filamentous microorganisms. These flocs and specially bacteria populations, were a source of vitamins, minerals and probiotics, but an important source of protein biomass that can contribute to cladoceran survival and density growth [9, 10]. It is important to mentioned that bacteria population in cladoceran culture not only was used as food, because they can use the nitrogen waste produced by organisms (cladocerans) to obtain their food to produce biomass, as well as, maintain water quality. Bacteria can be an essential source of vitamins and minerals, such as phosphorus [9, 10], and probiotics when those bacteria population were transformed to heterotrophic [8, 10].

That's why the main goal of this research was to study the use of bacteria produced in Biofloc system as only food to *S. vetulus* organisms cultured in laboratory in different temperatures to know if this nutritional source can support high densities of this cladoceran.

2. Materials and Methods

2.1 Sample of organisms

The strain of *S. vetulus* was obtained from a sample of 10 L from the culture ponds of Centro de Investigaciones Biológicas y Acuícolas de Cuemanco (CIBAC), México, in March of 2018. The sample was taken to the Laboratory of Live Food Production where it was filtrated through a 20 µm mesh. Organisms were concentrated in 1 L of water and observed in an optic microscope Leica ICC50 HD (10 and 20x). The specie was identified with the aim of key through images of Zooplankton (V.5.0) guide.

Adult organisms were collected in Petri plates (10 cm diameter) (5 per plate), in 20 mL of water and 2 mL of microalgae, for its culture. When the density reached to 2 org mL^{-1}, were placed in 1,000 mL of culture water, to subsequently pass it to 20 L of culture.

2.2 Experimental design

Nine plastic containers of 20 L were filled with 15 L of water with continuous light and aeration (Fig. 1). Three experimental temperatures were tested (19, 23 and 25 °C) each by triplicate and the organisms were fed with produced bacteria in a system culture of tilapia with Biofloc. Every day it was extracted 1 L of water from the cladocera culture and filled with 1 L of Biofloc culture, previously sieved through a mesh of 10 µm. Each third day, ten samples of 10 mL were taken, and all the organisms were counted to obtain an average (±S.D.).

2.3 Bacteria production in Biofloc system

A month before initiating the culture of *S. vetulus*, two plastic cylinders of 200 L were filled with 160 L of fresh water, with constant and strong aeration and a temperature of 25 °C. In the containers 35 tilapia juvenile stage were placed and were fed with the 5% of the organism's total weight with pellets (60% of protein), and 3% of the total weight of the organisms with coffee residuals as carbohydrates source.

2.4 Processing data

Values from each sampling (40), were introduced to a data base in Excel 2010 to obtain its descriptive statistic. Average values were extrapolated to 15 L of culture. Also, growth tendency curves were obtained for each cultured population at the different experimental temperatures.

Obtained values for each sampling were introduced in a Program of Life Tables made in Excel 2010 and therefore obtain the next reproductive parameters:

Reproduction rate: $Ro= \sum l_x * m_x$

Where:
$\sum$= sum
lx= Survival proportion to each phase
mx= produced organisms per each living organism of each phase

Intrinsic growth rate: $r= \log_e R_o/T_c$

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

Cohort generational time: $Tc= \sum x*lx*mx/Ro$

Where:
$\sum$= sum
lx= Survival proportion to each phase
mx= produced organisms per each living organism of each phase
Ro= reproduction rate

2.5 Statistical analysis

It was determined the existence of significant differences ($p<0.5$) between cladoceran culture tank with the experimental temperatures through an ANOVA test. When this analysis showed significant differences, it was proceeded to make a multiple media analysis through Tukey test. All this was done with the statistical program SYSTAT 13.0.

3. Results

Mean values (±S.D.) of the samplings in the three experimental temperatures are presented in Table 1. Where experimental temperature of 23 °C obtained the highest value with 334,956±437 organisms at 60 days of culture (22 org mL^{-1}), and the lowest was obtained in the temperature of 19 °C with 49,042±429 organisms (3 org mL^{-1}). The temperature of 25 °C obtained 136,802±408 organisms per 15 L culture tank (9 org mL^{-1}). The ANOVA showed significative differences ($p<0.001$) between the final density obtained by the three experimental temperatures.

Growth tendency curves are presented in Fig. 2. In all three cases, the tendency curve that best explained the growth was polynomic grade two. The R^2 values were up 0.98 in all tendency curves.

Table 2 show the reproductive values from the Life Table of each population in its respective experimental temperature. As it can be observed the best reproductive rate (Ro) was obtained at 23 °C with a production of 628 organisms per each reproductive event in the population and an r=0.16; while the lowest was obtained at 19 °C with only 91 produced organisms per reproductive event and an r=0.11. It should be noted that at a higher temperature (25 °C), the production of new organisms per female (256) and r=0.14 is lower, regarding 23 °C experiment. The Tc value were maintained between 39-40.

4. Discussion

It is important in the massive cladocera culture to consider the constant input of carbon and phosphorus [10], because it has been observed the change in the morphology of some populations of this crustacean. This condition can be modified when heterotrophic bacteria are aggregate to the culture as complementary food to the microalgae [10], which can be obtained by producing a tilapia culture based on Biofloc. It has been observed that these bacteria can enrich organisms with essential fatty acids (EFA), aminoacids, vitamins and pigments [11].

Very few works have been made with heterotrophic bacteria produced in a Biofloc system and used as cladocera food. One of them was with cladocera *M. australiensis* [12] which used the produced bacteria from the liquid digestion of pig manure, at different concentrations (10, 20, 30, 40, 50 and 60 mg L^{-1}) in culture containers of 50 L. The highest value of growth rate was found in the concentration of 30 mg L^{-1} with 0.15, similar value to the one found at temperature 23 °C in this investigation with an r=0.16. Castro *et al.* [13] that made a

similar experiment with *M. macrocopa*, also obtained higher densities at 23°C with 85,552±255 org $20L^{-1}$ with an r=0.13, different to this investigation which highest value obtained at that same temperature was of 334,956±437 org 20 L^{-1} and an r=0.16, observing that for *S. vetulus* it gave >300% being a better result the use of these bacteria to increase its population density.

Schwerin *et al*. [14], mention that cladocera, in massive cultures, show an adaptative plasticity to temperature conditions in cultures, due to the genetic pool of each population, which will affect the physiology biochemical reactions of the different cladocera populations, modifying their fecundity and reproduction of females [15]. To solve this problem, it is necessary to supply a diet high on proteins, easy to digest, so the difference found between *M. macrocopa* and *S. vetulus* is mainly due to the type of heterotrophic bacteria formed in the Biofloc, because is not the same, even though culture conditions are the same [16].

Brito *et al*. [17], mention that cell filtration and ingestion by *S. vetulus* fed with *Selenastrum capricornutum* and *C. vulgaris* is modified depending on the concentration of culture media. These authors found that *S. vetulus* presents a better ingestion rate when cell concentration is between 3-5 x 10^6 cel mL^{-1}. It is important to consider the size of the food particle, because when its higher the ingestion capacity of *S. vetulus* it is covered more quickly and therefore food concentration can decrease. When food particles concentration is adequate for *S. vetulus*, their nutritional requirements can be covered better and quicker and therefore make less effort to catch the food and thus spend less energy on getting it. In this specie of cladocera, it must be considered the increase of food concentration in the culture media, because organisms can present physiological and mechanical problems in filtration. Concentrations above 3 x 10^6 cell mL^{-1} can cause that *S. vetulus* filtration system stop being efficient or suffer damages.

Stark *et al*. [18], used the human liquid waste and urine with manure (cow/pig) in a *Daphnia pulex* culture of 10 L, obtaining densities of 468-1,236 org, with values of Ro between 6-18 org per female. Values below the ones obtained in this study with 167,478 org 10 L^{-1} and a Ro=628. Muñoz *et al*. [19], mention that not all cladocera species respond equally in their density population growth, due to the differences in the supplied food. These differences are mainly given in their obtained reproductive values in Life Tables.

Regarding to genus *Simocephalus* sp., Chan *et al*. [20], worked with *S. mixtus* fed with *Chlorella* sp. and *Scenedesmus* sp., found that the value of Ro increases when temperature does from 20°C to 25°C, but with values of 35 org per female, different to this study where the lowest value of Ro was of 91 org per female. Arias *et al*. [21] (2013), that worked with cultures of *D. ambigua* and *S. serratus* fed with *Chlorella* sp. and *Pseudokirchneriella subcapita* at a concentration of 1.05 x10^8 cell mL^{-1}, as well as with yeast (0.5 mL L^{-1} of culture), had values of Ro between 37 to 43 org per female, values below the ones in this study with a lower density of organisms (49,042±429) and a Ro=91. Fernandez *et al*. [22] that cultured *S. vetulus* with *Scenedesmus acutus* (1 x 10^6 cel mL^{-1}) in addition to *Macrocystis aeruginosa*, obtained growth rates (r) with values between 0.21-0.30. Both values higher tan the ones obtained in this study with an r between 0.11 to 0.16.

Grossart *et al*. [23], mention that the use of bacteria can be efficient, because of its size they can be ingested and hosted in the digestive tract, even though they can not be considered with a high nutritional value for zooplankton. Bacteria as food source must be consider as another protein source, but not the only source. Even though its strongest potential is that bacteria that can reproduce in a cladocera culture system, be used as a cleaning system of water to eliminate the exoskeleton of dead organisms, as well as the nitrogenous wastes produced by the organisms. It is sought that the increase in bacterial density in the culture really works as a nutrient recycling system that can be used by phytoplankton, which be use as the main nutrient in the diet of these crustaceans [24].

It is important not to forget the genetic compound that each specie and population of cladocera have, which makes the response that each population has more efficient to the variables of space, type of food and different culture temperatures [25]. Bacteria as a food source for cladocera, can play an important role in the different species and populations of this organism, but in the case of *S. vetulus*, even though it presented higher values than the study with *M. macrocopa* [13], it maintains low growth rates, so a complementary diet must be used, together with the supplied microalgae, green or brown. Produced heterotrophic bacteria by a Biofloc system can be considered as a vitaminic, mineral and enzymes supply, which are produced by the bacteria and can benefit the cladocera in culture.

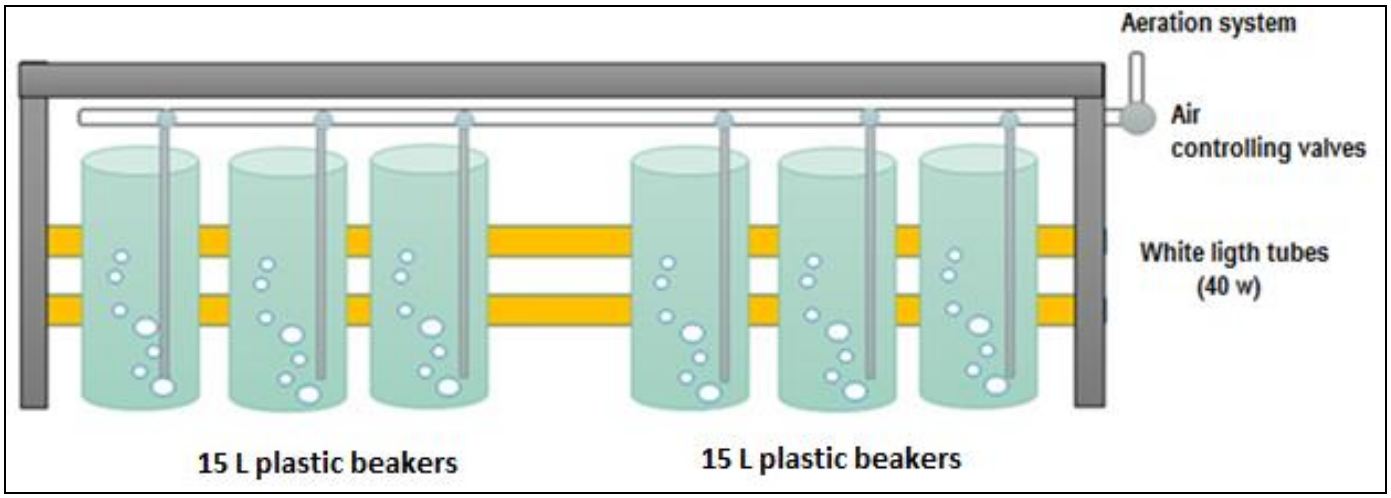

Fig 1: Experimental design of *S. vetulus* at the three experimental temperatures (19°, 23° and 25°C) fed with heterotrophic bacteria produced in a Biofloc system

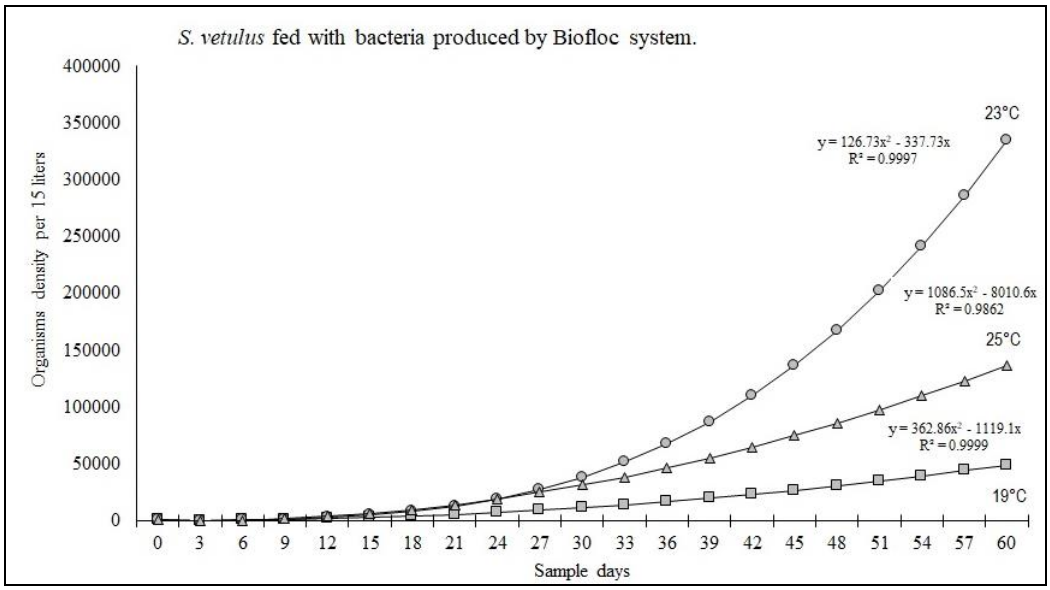

Fig 2: Population growth tendency curves in the three experimental temperatures.

Table 1: Mean values (±S.D.) of *S. vetulus* density in each experimental temperature.

Sampling	Experimental temperature		
	19 °C	23 °C	25 °C
0	533±11	533±11	533±11
3	426±24	378±37	187±18
6	587±20	765±26	365±23
9	1,013±113	1,445±154	1,657±157
12	1,707±15	2,706±15	3,689±26
15	2,666±126	4,832±183	6,460±146
18	3,892±123	8,109±190	9,972±199
21	5,385±315	12,823±323	14,223±342
24	7,144±147	19,261±126	19,213±192
27	9,170±179	27,708±127	24,944±149
30	11,462±416	38,449±483	31,414±431
33	14,021±402	51,771±415	38,624±462
36	16,846±184	67,959±179	46,574±157
39	19,938±199	87,300±173	55,263±263
42	23,296±232	11,0078±278	64,692±269
45	26,921±126	13,6581±158	74,861±186
48	30,812±128	167,093±197	85,770±177
51	34,970±397	201,901±320	97,418±341
54	39,394±439	241,290±429	109,806±498
57	44,085±508	285,547±547	122,934±593
60	49,042±429^{c}	334,956±437^{a}	136,802±408^{b}

Note: Different letter at final raw, show significant differences (p<0.001).

Table 2: Reproductive values of *S. vetulus* population density in the three experimental diets.

Experimental temperature	Reproduction rate (Ro)	Cohort generational time (Tc)	Growth rate (r)
19 °C	91	39.37	0.11
23 °C	628	40.92	0.16
25 °C	256	39.33	0.14

5. Conclusion

The use of heterotrophic bacteria as only source of food for *S. vetulus*, does not provide the nutrient quantity to obtain high culture density, but it can be used as complement for the microalgae diet. These heterotrophic bacteria contribute with other products like: essential fatty acids, vitamins, pigments, enzymes, and low protein quantity, that can be used as complement diet to *S. vetulus*. Produced bacteria in a Biofloc culture, can be used when microalgae are not available in high concentrations or microalgae cultures fall.

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