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Population density and reproductive potential comparison of *Simocephalus vetulus* (Müller, 1776) cultured with a mixed diet with chlorophytes (*Scenedesmus* sp + *Chlorococcum* sp.) and diatoms (*Pinnularia* sp.) at different concentrations

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Abstract

This study was focused to know the population density growth of *Simocephalus vetulus* fed with four experimental diets using chlorophytes and diatoms during 60 days of culture, in 12 L plastic beakers filled with 10 L of water, with an initial density of 20 organisms. Diets were: 1) Chlorophyte 100%, 2) Diatoms 100%; 3) Chlorophytes 25% and Diatoms 75%, and 4) Chlorophytes 75% and Diatoms 25%. Organisms fed with Chlorophytes 75% and Diatoms 25% obtained the highest density with $6,041 \pm 18$ org $10L^{-1}$, while the lowest density value was for Chlorophytes 100% with $1,109 \pm 10$ org $10L^{-1}$. Highest value of R_0 was obtained with diet Chlorophytes 75% and Diatoms 25% with $1,287$ org female $^{-1}$, $T_c = 16.15$ days, and $r = 0.43$. Lowest reproductive potential values were shown in Chlorophyte diet with 148 org female $^{-1}$, $T_c = 21.98$ days, and $r = 0.23$. ANOVA and Tukey test shown significant differences ($P < 0.05$) between all treatments. For *S. vetulus* cladoceran specie, it is necessary to make cultures using mixed diets with Diatoms and Chlorophytes microalgae to obtain better density results in laboratory conditions.

Keywords: *Simocephalus vetulus*, chlorophytes, diatoms, population density, life table

1. Introduction

Cladocera group are small size crustaceans (2mm length) with high population growth rates, principally from freshwaters but some species have invaded marine environments [1]. These microcrustaceans are a monophyletic group and are an important component of zooplankton community of epicontinental systems. Until today, approximately 620 species are registered and 200 of those species are found at Neotropical region. These organisms are found in all continental systems from tropical latitudes to boreal regions [2].

Cladoceran group not only highlights because of their parthenogenetic reproduction and their short life cycle, but also, for their capacity to reproduce in short periods. Also, are an important source of amino acids, lipids, essential fatty acids, vitamins, and enzymes (peptidases, proteinases, lipases, and amylases) that are used like exoenzymes in the intestinal tract of fish larvae [3]. Within plankton organisms, cladoceran group represent an important compound in trophic chain of many water bodies, and between organism's variety, that conform the zooplankton. Cladoceran group is a vulnerable group for predation and are used as adequate prey for fish and crustacean larviculture and aquarium-hobby [4-8].

Cladoceran culture offers an advantage to generate a great number of organisms in short time periods, if producers can maintain temperature, water quality, and food in optimal conditions [9]. Sarma *et al* [10] and Peña-Aguado *et al*. [11] mentioned that quantity and nutritional value of microalgae affect the reproduction rate and frequency of cladocerans and rotifers, and their relationship between parthenogenetic eggs and amount of available food [6].

Cladoceran usefulness in aquaculture and aquarium-hobby lies that this organism shows a great diversity, their nutritional value can be modified because they are filter feeders and are easily prey for fishes and crustaceans' larvae stages [4, 7, 12, 13]. That is why the main goal of this study was to evaluate the effect of population growth density and their reproductive potential of *S. vetulus*, using four experimental diets (chlorophytes and diatoms in different concentrations)

2. Materials and methods

2.1 Obtaining organisms strain

S. vetulus organisms were obtained from 10 L sample taken out from the 10,000 L ponds of Centro de Investigaciones Biológicas y Acuícolas de Cuernavaca (CIBAC), Universidad Autónoma Metropolitana Xochimilco. The sample was taken to Live Food Production and Biofloc Laboratory where it was filtered with a 20 µm mesh. The organisms were concentrated in 1 L and observed in an Optical Microscope Leica ICC50 HD (10 and 20x). The cladoceran specie was identified with an Imagen Zooplankton Key (V.5.0).

S. vetulus organisms in adult stage (five organisms) were placed in Petri dish (10 cm diameter), with 20 mL of water and 2 mL of microalgae. Twenty-five replicas were done. When density reached 2 org mL⁻¹, the organisms were placed

in 1 L of water and then when they reached a density of 2 org mL⁻¹ again, organisms were placed in 20 L culture beakers.

2.2 Experimental design

Twelve 20 L plastic beakers were filled with 10 L of freshwater with continuous light and aeration (Fig.1). Four experimental diets were tested: 1) Chlorophytes 100%; 2) Diatoms 100% 3) Chlorophytes 25% and Diatoms 75%; and 4) Chlorophytes 75% and Diatoms 25%. Each diet was made by triplicate. Every day 1 L of culture medium was extracted and added 1 L of microalgae diet. Every third day, three samples of 10 mL were taken, and all organisms were counted to obtain their mean value (±SD). The four-density experiment began with a density of 20 org 10L⁻¹ during 60 culture days.

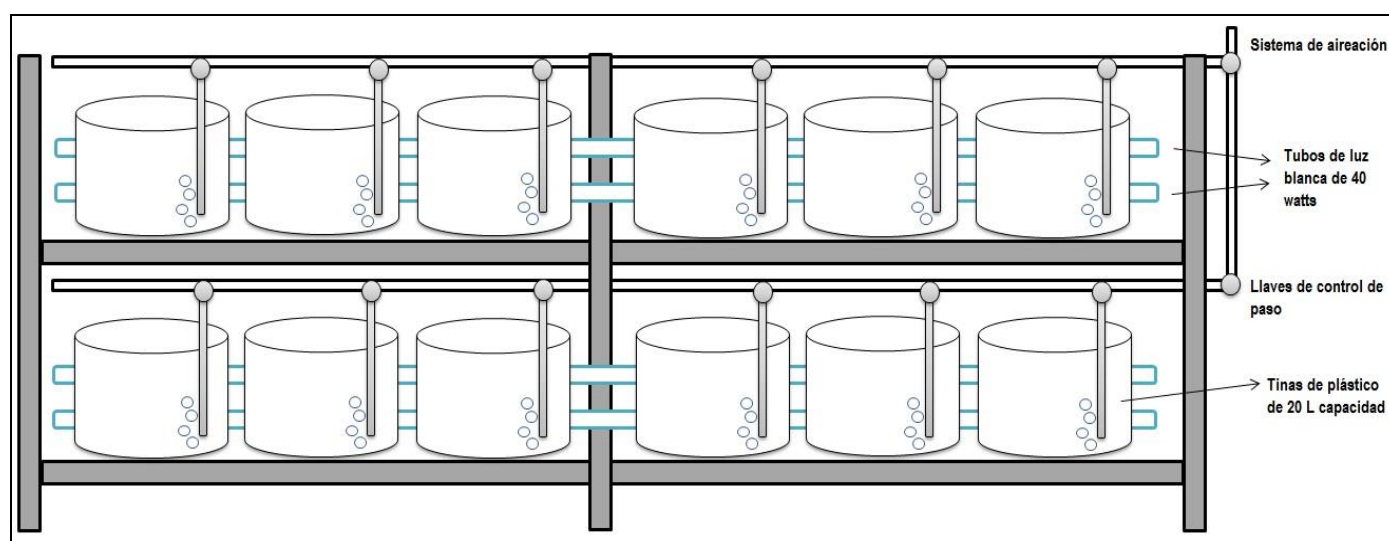


Fig 1: Experimental design used to culture *S. vetulus* with four experimental diets.

2.3 Information processing

All sample values from each experimental diet were introduced in an Excel 2010 database to obtain their descriptive statistics. The values were extrapolated to 10 L. Also, density tendency curves were made from each experimental diet.

These values were introduced in a Life Table program made in Excel 2010 to obtain the following reproductive parameters:

$$\text{Reproduction Rate: } R_0 = \sum l_x * m_x$$

Where:

\sum = summary

l_x = survival proportion for each phase

m_x = produced organisms for each live organism from each phase

$$\text{Intrinsic Growth Rate: } r = \log R_0 / T_c$$

Where:

$\log R_0$ = logarithm of natural reproduction rate

T_c = generational time of cohort

$$\text{Generational Time of cohort: } T_c = \sum x * l_x * m_x / R_0$$

Where:

\sum = summary

l_x = survival in each phase

m_x = organisms produced in each phase

R_0 = reproduction rate

2.4 Statistical analysis

ANOVA test was made to determine significant differences ($P < 0.05$) between population density from each experimental diet. When significant differences were shown in ANOVA analysis, a multiple mean comparison test was made with Tukey technique. This analysis was made in Statistical Program SYSTAT 13.0.

3. Results

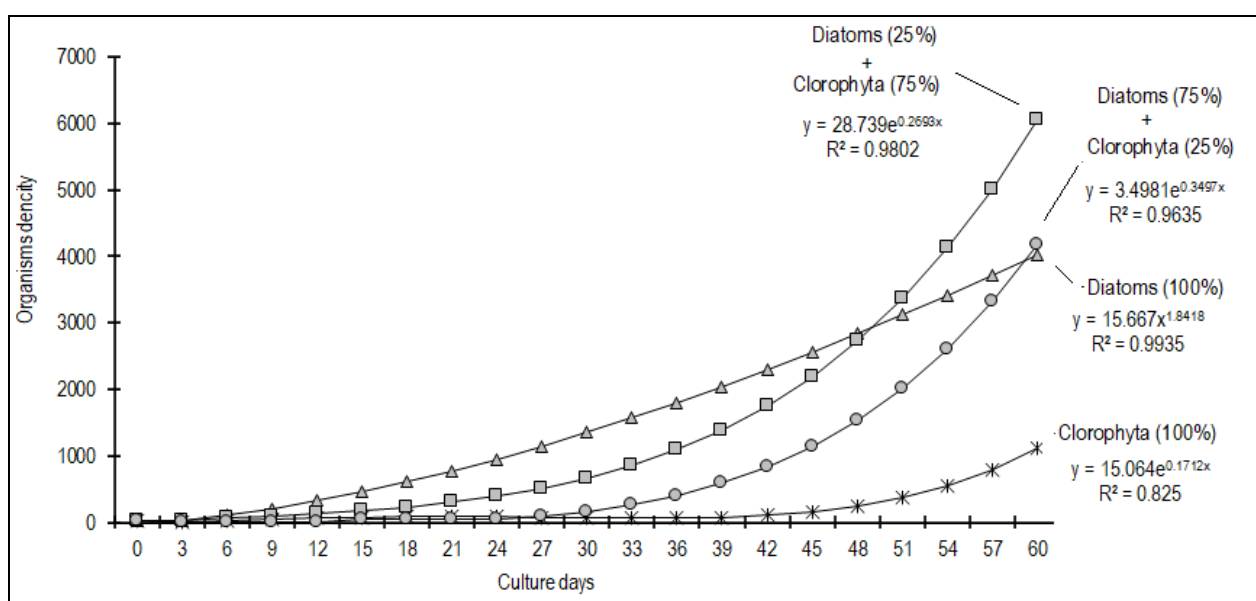
Density mean values (±S.D.) of *S. vetulus* from each experimental diet are shown in Table 1. Meanwhile, Fig.2 shows density tendency curves. Only Diatom 100% curve shows potential curve type, other three, show an exponential curve type.

Table 1: Mean values (\pm SD) of population density of *S. vetulus* fed with four experimental diets at 10 L culture medium.

| Sampling days | Diatoms (100%) | Chlorophytes (100%) | Diatoms (25%) + Chlorophytes (75%) | Diatoms (75%) + Chlorophytes (25%) |
|---------------|----------------|---------------------|------------------------------------|------------------------------------|
| 0 | 20 \pm 2 | 20 \pm 3 | 20 \pm 3 | 20 \pm 2 |
| 3 | 36 \pm 2 | 10 \pm 4 | 31 \pm 4 | 4 \pm 1 |
| 6 | 108 \pm 18 | 26 \pm 6 | 62 \pm 2 | 7 \pm 1 |
| 9 | 204 \pm 17 | 44 \pm 2 | 96 \pm 10 | 8 \pm 1 |
| 12 | 321 \pm 20 | 61 \pm 10 | 133 \pm 14 | 13 \pm 2 |
| 15 | 455 \pm 20 | 74 \pm 5 | 178 \pm 17 | 37 \pm 5 |
| 18 | 606 \pm 14 | 82 \pm 11 | 232 \pm 18 | 50 \pm 5 |
| 21 | 773 \pm 13 | 84 \pm 15 | 302 \pm 14 | 53 \pm 14 |
| 24 | 953 \pm 10 | 82 \pm 14 | 392 \pm 17 | 53 \pm 12 |
| 27 | 1147 \pm 11 | 76 \pm 6 | 508 \pm 13 | 97 \pm 10 |
| 30 | 1353 \pm 28 | 69 \pm 6 | 657 \pm 50 | 165 \pm 16 |
| 33 | 1572 \pm 20 | 64 \pm 5 | 847 \pm 60 | 264 \pm 60 |
| 36 | 1803 \pm 60 | 65 \pm 3 | 1086 \pm 12 | 402 \pm 14 |
| 39 | 2044 \pm 15 | 78 \pm 3 | 1383 \pm 16 | 587 \pm 60 |
| 42 | 2297 \pm 17 | 108 \pm 8 | 1748 \pm 11 | 831 \pm 90 |
| 45 | 2560 \pm 10 | 163 \pm 15 | 2193 \pm 13 | 1143 \pm 17 |
| 48 | 2833 \pm 17 | 250 \pm 14 | 2729 \pm 13 | 1535 \pm 17 |
| 51 | 3116 \pm 11 | 377 \pm 50 | 3367 \pm 18 | 2020 \pm 11 |
| 54 | 3409 \pm 10 | 556 \pm 11 | 4123 \pm 19 | 2612 \pm 10 |
| 57 | 3711 \pm 17 | 796 \pm 16 | 5009 \pm 15 | 3325 \pm 10 |
| 60 | 4023 \pm 19 | 1109 \pm 10 | 6041 \pm 18 | 4174 \pm 20 |

In all experiments, the organisms survived during all 60 culture days. After that day, all organisms died quickly to zero density. Diatoms 25% and Chlorophyte 75% diet obtained the

highest density with 6,041 \pm 18 org 10 L⁻¹, and the lowest value was shown in Chlorophyte 100% diet with 1,109 \pm 10 org 10 L⁻¹.

**Fig 2:** Density tendency curves of *S. vetulus* cultured at four experimental treatments

ANOVA analysis and multiple mean comparison by Tukey test technique shown significant differences between all treatments ($P < 0.05$), using the last density sample (60 culture days) when they reach highest density

Reproductive values are shown in Table 2. It can be observed that highest R_o value was obtained in Diatoms 100% and Diatoms 75%+Chlorophyta 25% with values of 1,060 and

1,287 organisms female⁻¹ respectively. These diets presented T_c values of 16.15 and 18.86 days respectively and r value of 0.43 and 0.38, respectively. The lowest reproductive potential values were shown in diet with absence of diatoms source. Chlorophyta 100% diet presented a $R_o = 148$ org female⁻¹, a $T_c = 21.98$, and $r = 0.23$.

Table 2: Reproductive values in *S. vetulus* population, fed with the four experimental diets

| Experimental diets | Reproduction rate per female (R_o) | Generational time of cohort (T_c) | Population growth rate (r) |
|----------------------------------|--|---------------------------------------|--------------------------------|
| Chlorophytes (100%) | 148 | 21.98 | 0.23 |
| Diatoms (100%) | 1,060 | 16.15 | 0.43 |
| Chlorophytes 25% and Diatoms 75% | 1,287 | 18.86 | 0.38 |
| Chlorophytes 75% and Diatoms 25% | 815 | 19.98 | 0.34 |

4. Discussion

Food selection was one of the principal variables which can modify and control the population density in zooplankton culture medium, because it affects directly the life stage of zooplankton. That is why the food composition, digestibility and size of microalgae used as food to these organisms can be a bottleneck to determine the growth rate and population density of this important organisms used to fed larvae stages in aquaculture and aquarium-hobby systems [14]. In this study, the great difference between densities can be seen at 60 culture days between Diatoms 100% and Chlorophytes 100% diets in *S. vetulus*, because they show a difference of 3,000 org 10L⁻¹. Nevertheless, the highest values have a difference of 2,000 org 10L⁻¹ and 5,000 org 10L⁻¹ respectively (Chlorophytes 25% and Diatoms 75% diet with 6,041±18 org 10L⁻¹). This is important because with *S. vetulus* specie is important to supply a mixed diet with diatoms and chlorophytes to improve better density growth in culture medium in laboratory conditions. A monospecific with chlorophytes diet to *S. vetulus* cannot supply all nutritional requirements that this cladoceran need to get better density growth [3].

Microalgae in zooplankton cultures can be considered as the principal food source because of their nutritional value. Microalgae have a great capacity to synthesize and amass great quantities of polyunsaturated fatty acids (PUFA). Green microalgae have highest quantities of ω3 PUFA [15], but their low content of high unsaturated fatty acids (HUFA) is a limiting factor to cladoceran, because their consequences in this group is a low-density growth and reproduction rates. That is why some authors mentioned that is important to use mixed microalgae diets in cladoceran groups because each microalgae group supply different nutritional components or another food types like bacteria and yeast, can supply some vitamins like B complex or probiotics [3]. Diatoms have demonstrated their high content of polyunsaturated fatty acids and principally eicosapentaenoic acid (EPA) which can get 7-34% of dry weight from each cell [16]. This was demonstrated by the study with *D. pulicaria* [17]. These authors findings show that feeding this cladoceran with diatom microalgae was better than those experiments cultured with green microalgae. Our study confirms that a diet with diatoms principally or mixed diets with diatoms 75% and Chlorophytes 25% were better than those diets only with green microalgae. In the case of *S. vetulus* in a monospecific diet even a diatoms source, need a supply of 25% or 75% of Chlorophytes to obtain better results. Studies with *Diaphanosoma birgei* [18] cultures fed with *Ankistrodesmus gracilis* (Chlorophyta) obtained a $r = 0.18$. This value is lower with respect to the lowest values obtained in this study ($r = 0.23$ with Chlorophytes 100%). This confirm that monospecific diet with chlorophytes microalgae is less efficient to increase population density growth rate in cladoceran culture.

The low efficiency of chlorophytes can be due to its low size compared to diatoms. In this study it was used *Pinnularia* sp. which can have 100-280 μm length with respect to *Scenedesmus* sp. and *Chlorococcum* sp., that only reach 15-20 μm and 10-20 μm length respectively [19, 20]. In case of herbivorous zooplankton like cladoceran group prefer higher length microalgae like diatoms [21, 22]. That is why cladocerans fed with diatoms or chlorophytes mixed with diatoms obtained better results, because their filtration system and filtration efficiency were better with larger microalgae and their nutritional need can be cover better [23]. But, authors like

Lee y Ban [24] mentioned that an exponential or potential increase of density in cladoceran can provoke a decrease of density growth and reproduction of females. Nevertheless, in this study was different, Diatoms 100% or Diatoms 75% diets shown r values of 0.43 and 0.38 respectively and their R_0 value of 1,060 and 1,287 org female⁻¹.

5. Conclusion

Therefore, it is necessary to make *S. vetulus* culture in laboratory with mixed diets of Chlorophytes and Diatoms because their nutritional supply is covered better than if a monospecific microalgae source is used. A mixed diet allows better density growth and reproduction rates in laboratory conditions and producers can obtain a biomass resources more quickly and can use it as live food directly to larvae stage of fishes and crustaceans or use it as dried biomass to make food pellets or flakes.

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