Development of *Moina minuta* (Hansen, 1899) (Cladocera: Anomopoda: Moinidae) under different food sources

Desenvolvimento de *Moina minuta* (Hansen, 1899) (Cladocera: Anomopoda: Moinidae) submetida a diferentes fontes alimentares

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ABSTRACT  
The aim of this study was to analyze the population growth of the cladoceran *Moina minuta* using three food sources: *Chlorella* sp., *Saccharomyces cerevisiae* and fish broth. The experimental design was completely randomized with 3 treatments and 3 replicates, being the experiment carried out for 12 days. *M. minuta* fed with *Chlorella* sp. showed maximum population density of 11.27 org. mL⁻¹ on the ninth day of experiment, while the organisms fed with *S. cerevisiae* and fish broth reached 4.92 and 3.67 org. mL⁻¹ on
the eighth and third day of experiment, respectively. Cladocerans fed with *Chlorella* sp., *S. cerevisiae* and fish broth showed, respectively, growth velocity of 0.95, 0.44 and 0.28 org. mL$^{-1}$ day$^{-1}$, specific growth rate of 0.29, 0.24 and 0.24 day$^{-1}$, and doubling time of 2.48, 2.94 and 3.20 day$^{-1}$. It was verified that *M. minuta* reached high values of the population growth variables, therefore, this species is viable for production on a large scale. Among the three treatments analyzed, the specimens fed on *Chlorella* sp. showed the best growth variable averages.

**Keywords:** aquaculture, cladoceran, *Chlorella* sp., fish broth, *Saccharomyces cerevisiae*, population growth.

**1 INTRODUCTION**

*Moina minuta* (Hansen, 1899) (Figure 1) is a pelagic cladoceran species with a size between 0.5 and 0.7 mm. It is distributed in freshwater environment in Brazil (ELMOOR-LOUREIRO, 1997). This species has characteristics that make it promising for use as live food in aquaculture, such as asexual reproduction by parthenogenesis (MARTÍNEZ-JERÓNIMO; RODRÍGUEZ-ESTRADA; VILLASEÑOR-CÓRDOVA, 2007), allowing high population growth rates, resistance to physical-chemical variations of water, dominance among the zooplankton taxa both in natural environment (REJAS et
and in culture and good acceptability as live food by larvae of cultivable aquatic animals (LIMA et al., 2017; RAMOS; ABE; FUJIMOTO, 2016).

Figure 1. Parthenogenetic female of *Moina minuta* (4x microscope).

Feeding corresponds to one of the important points for biomass production. Cladocerans feed on a range of food resources, depending on their distribution and adaptations. Anomopods such as the organisms of the genus *Moina* are filter feeders, feeding on microalgae, ciliated protists, bacteria and organic detritus (KUMAR; HWANG, 2008; SMIRNOV, 2017). Microalgae are considered good source of food for cladocerans, especially chlorophytes because they are nutritionally adequate for many zooplankton species (PEÑA-AGUADO; NANDINI; SARMA, 2005; UDDIN et al., 2022). *Chlorella sp.* is a chlorophyte species suitable for cladoceran culture because it contains vitamins, minerals, and high protein concentration reaching about 60%, besides containing about 18 essential amino acids (RAJI et al., 2018). Another advantage of this microalgae is the size between 4 and 5 µm, which facilitates capture and ingestion (PEÑA-AGUADO; NANDINI; SARMA, 2005).

Other food sources such as yeast (*Saccharomyces cerevisiae*) (Meyen ex Hansen, 1838) are widely researched for zooplankton feeding, as they are sources of vitamins, polysaccharides, proteins and have a size that varies from 4 to 5 µm. In addition, this food source is of easy preparation and have affordable prices, not requiring cultivation
Preliminary research has been looking for the use of fish waste that are usually not suitable for human consumption, these wastes include all parts that are not consumed, such as bones, viscera, head and tails, the main use of these inputs are destined to fish meal production for animal feed or discarded (KRISTINSSON; RASCO, 2000; MO; MAN; WONG, 2018). These rejects can be reused as an alternative medium for M. minuta cultivation, thus contributing to the environment and reducing the production costs of this cladoceran.

Analyzing the best management strategy, in terms of food, to obtain a higher population density of M. minuta is important for aquaculture production, especially the larval stage, since during this life stage the animals depend basically on live food for their development. Thus, the objective of the present study was to analyze the population growth of the cladoceran M. minuta using three food sources: i) Chlorella sp., ii) S. cerevisiae and iii) Fish broth.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

The experiment lasted for 12 days and the M. minuta organisms were distributed in the experimental units one day before the start experiment, considered day 0. The experimental design was completely randomized with 3 treatments consisting of different food sources (Chlorella sp., Saccharomyces cerevisiae and fish broth) and 3 replicates per treatment. The experimental units were continuously aerated and artificially illuminated with fluorescent light in a photoperiod of 12 h of light and 12 h of dark. The experimental units contained a useful volume of 1 liter. The initial density of Moina minuta on day 0 corresponded to 0.2 M. minuta mL⁻¹ per experimental unit. Every day, the total water exchange and addition of new culture medium was performed.

2.2 PREPARATION OF CULTURE MEDIA

Chlorella sp. was cultured in a static system using as culture medium the agricultural fertilizer NPK (nitrogen: phosphorus: potassium - 20:5:20) at a concentration of 0.35g L⁻¹ (SIPAÚBA-TAVARES; ROCHA, 1993). The microalgae culture received
constant aeration and was continuously illuminated with fluorescent light during the experiment period. *Chlorella* sp. was offered to *M. minuta* at a concentration of approximately $9 \times 10^4$ cells mL$^{-1}$. The experimental units' algal density was consistently replenished each day and monitored using a Neubauer chamber for cell counting.

The commercial lyophilized yeast *S. cerevisiae* was offered to *M. minuta* at a concentration of 1.25 mg L$^{-1}$ (OCAMPO; BOTERO; RESTREPO, 2010). The fish broth was produced by cooking fish waste and thereafter added to the *M. minuta* culture at a concentration of 20 mL L$^{-1}$.

### 2.3 CULTIVATION CONDITIONS

The water quality of the experimental units was measured every three days using a multiparameter probe (Horiba U-10, USA). Dissolved oxygen levels ranged from 7.28 ± 1.25 mg L$^{-1}$, while the pH averaged 8.02 ± 0.13. The temperature was approximately 29.21 ± 0.15°C, and the electrical conductivity was 0.49 ± 0.04 mS cm$^{-1}$ throughout the experiment.

### 2.4 MONITORING THE POPULATION GROWTH OF *Moina minuta*

Daily, at the same time, a sample of 20 mL of the *M. minuta* population from each experimental unit was collected and counted, in a Petri dish under a trinocular stereomicroscope (Axioskop 40, Zeiss, USA), the total amount of organisms, except for day 0, where there was not counting. After the experiment, with the data obtained from the daily counts, the population growth variables of *M. minuta* were calculated using the methodology of Prieto, Cruz and Morales (2006): maximum density of organisms (MDO), day of maximum density (DMD), growth velocity ($GV = (FC – IC) / (FT – IT)$), specific growth rate ($K = (\ln FC – \ln IC) / (FT – IT)$) and doubling time ($DT = \ln 2 / K$). Where: 

- $\ln$ is the natural log; 
- IC is the initial concentration of organisms in the culture; 
- FC is the final concentration of organisms in the exponential phase of population growth; 
- FT is the final time of the experiment and 
- IT is the initial time of the experiment.
2.5 STATISTICAL ANALYSIS

The data obtained were submitted to the Shapiro-Wilk and Bartlett tests, where it was found that they presented normal distribution and homoscedasticity of the variances. Subsequently, the data were submitted to one-way analysis of variance (ANOVA) to compare the effect of the different food sources (Chlorella sp., S. cerevisiae and fish broth) on the population growth variables of M. minuta (MDO, DMD, GV, K and DT) during the 12 days of the experiment. When significant F values were recorded in the ANOVA, Tukey’s multiple comparisons test was used to discern significant differences between treatments (ZAR, 2010). All analyses were performed using the Software ASSISTAT 7.7, with α = 0.05 (SILVA; AZEVEDO, 2016).

3 RESULTS

During the first day of the experiment, similar population growths of M. minuta were observed in all treatments. However, on the second day, there was population increase in all treatments, to a greater value for the organisms fed with Chlorella sp. and S. cerevisiae (Figure 2). The peak of population growth of M. minuta fed with fish broth was recorded on the third day (3.67 ± 0.48 org. mL⁻¹) and fed with S. cerevisiae on the eighth day (4.92 ± 0.51 org. mL⁻¹), while the organisms fed with Chlorella sp. showed two peaks of population growth, occurring on the fourth and ninth day of experiment, being the latter the highest (11.27 ± 2.48 org. mL⁻¹; F = 26.75, p < 0.05). In the period between the tenth and twelfth day of experiment, population declines occurred in practically all treatments (Figure 2, Table 1).
Figure 2. Population growth of *Moina minuta* (org. mL⁻¹) fed with *Chlorella* sp., *Saccharomyces cerevisiae* and fish broth during 12 days of experiment. * = significant at p < 0.05.

The results of the population growth variables of *M. minuta* indicated significant influence (p < 0.05) of the type of food on the values of MDO (F = 14.95) and GV (F = 24.30), where the highest values occurred in the treatment with *Chlorella* sp. (Table 1). Conversely, there was no influence (p > 0.05) of the type of food on the values of K GV (F = 0.58) and DT (F = 0.78; Table 1).

Table 1. Means (±SD) of the growth variables of *Moina minuta* fed *Chlorella* sp., *Saccharomyces cerevisiae* and fish broth.

<table>
<thead>
<tr>
<th>Food sources</th>
<th>MDO (org. mL⁻¹)</th>
<th>DMD (day)</th>
<th>GV (org. mL⁻¹ day⁻¹)</th>
<th>k (day⁻¹)</th>
<th>DT (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlorella</em> sp.</td>
<td>11.27±2.48ᵃ</td>
<td>9ᵃ</td>
<td>0.95±0.16ᵃ</td>
<td>0.29±0.06ᵃ</td>
<td>2.48±0.49ᵃ</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>4.92±0.51ᵇ</td>
<td>8ᵇ</td>
<td>0.44±0.04ᵇ</td>
<td>0.24±0.01ᵇ</td>
<td>2.94±0.18ᵇ</td>
</tr>
<tr>
<td>Fish broth</td>
<td>3.67±0.48ᵇ</td>
<td>3ᵇ</td>
<td>0.28±0.05ᵇ</td>
<td>0.24±0.08ᵇ</td>
<td>3.20±0.86ᵇ</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.05***</td>
<td></td>
<td>&lt;0.01***</td>
<td>0.59**</td>
<td>0.49***</td>
</tr>
<tr>
<td>CV (%)</td>
<td>27.59</td>
<td></td>
<td>22.28</td>
<td>27.57</td>
<td>24.81</td>
</tr>
</tbody>
</table>

MDO (maximum density of organisms), DMD (day of maximum density), GV (growth velocity), k (specific growth rate), DT (doubling time), CV (coefficient of variation). The significance level of influence of the type of food on the population growth variables of *M. minuta* by Tukey’s test at 0.05% probability are described as: NS = Not significant, * = significant at p < 0.05, ** = significant at p < 0.01. Source: Authors.
The organisms fed with *Chlorella* sp., *S. cerevisiae* and fish broth showed GV value of 0.95, 0.44 and 0.28 org. mL⁻¹ day⁻¹, K value of 0.29, 0.24 and 0.24 day⁻¹, and DT of 2.48, 2.94 and 3.20 day⁻¹, respectively (Table 1).

4 DISCUSSION

The population growth of *M. minuta* showed better results with the use of *Chlorella* sp. as food, corroborating with the studies of Peña-Aguado, Nandini and Sarma (2005). These authors observed that the cladocerans *Ceriodaphnia dubia* (Richard, 1894) and *Moina macrocopa* (Straus, 1820) and the rotifers *Brachionus calyciflorus* (Pallas, 1766) and *Brachionus rubens* (Ehrenberg, 1838) presented better results of increasing density throughout the experiment using as food the chlorophytes *Chlorella vulgaris* (Beijerinck, 1890) and *Scenedesmus acutus* (Meyen, 1829) in relation to the use of only *S. cerevisiae*, with the latter providing the worst results of population growth.

Chlorophytes such as *Chlorella* sp. represent the main food source of freshwater zooplankton because of their excellent nutritional values such as high protein, vitamins, minerals and unsaturated fatty acids C18, mainly linoleic (RAJI et al., 2018). Yeast, such as *S. cerevisiae* is rich in vitamins, polysaccharides, proteins and nucleotides, which makes them an important food source in aquaculture (SHEYKHI et al., 2019), and its use as a complement with microalgae can make the food more complete for zooplankton development. However, yeast is not suitable as sole food in zooplankton culture, as it does not contain all the nutrients that these organisms need for its development, such as low levels of essential fatty acids (OTERO et al., 2013; PEÑA-AGUADO; NANDINI; SARMA, 2005).

Organic matter such as fish broth are sources of proliferation of bacteria and other microorganisms that serve as food for zooplankton, however Smirnov (2017) reported that some species of bacteria such as *Pseudomonas* and *Hydrogenophaga* are toxic to cladocerans, which could harm the population development of *M. minuta*. In addition, feeding only on bacteria is not adequate as a nutritional source for cladocerans due to nutritional deficiencies, such as the absence essential fatty acids and sterols (TAIPALE et al., 2014). Suminto et al. (2019) evaluated the inclusion of fermented organic matter
(tofu waste, rice bran and fish meal) together with *Tetraselmis chuii* (Butcher, 1959) in the growth of *Diaphanosoma brachyurum* (Liévin, 1848), and suggested that the inclusion of fermented organic matter in a proportion greater or less than 50% in relation to microalgae impaired the population development of this cladoceran. There is no mention in the literature of studies that address the use of broth originated from the cooking of fish waste as food for zooplankton, so further studies are needed to determine the technique of producing fish broth and the level of its inclusion in zooplankton culture, because the addition in low proportion in cultivation may be insufficient for feeding, but high proportion of addition of waste can promote eutrophication of the culture medium stimulating the proliferation of pathogenic bacteria.

During the initial stages of the experiment, all treatments exhibited low organism density and high food availability. This resulted in rapid population growth due to parthenogenetic reproduction until the population peaked.

With the increase in density of organisms, the food concentration is no longer sufficient, causing the death of the cladocers and the alternation to sexual reproduction strategy, and consequently decrease of the population. The peak of population growth of cladocers represents the ideal moment of collection for use as live food in aquaculture.

In this study, *M. minuta* fed with *S. cerevisiae* had a lower MDO than specimens fed with *Chlorella* sp. Similar responses were obtained by Otero et al. (2013) and Nakauth et al. (2015) with other Cladocera, although their results showed lower MDO values. These variations could be linked to the inherent characteristics of the Cladocera species used in each experiment, since they have different life strategies, such as lifespan, sexual maturity, reproduction, feeding, among others, so they respond differently to environmental variations and food sources (STARK; BANKS; VARGAS, 2004).

The present study showed that the cladocers reached MDO (ninth, eighth and third day) when they were fed with *Chlorella* sp., *S. cerevisiae* and fish broth, respectively. This result may be associated with the nutritional value of the food sources, because the higher the population peak, the longer it takes to reach it. Another factor that influences the population development of cladocers is environmental variation, such as temperature. In the study by Nakauth et al. (2015), the MDO occurred on the seventh day.
with the temperature around 25°C, and in the present study average temperatures of 29.21 ± 0.15°C were observed. Higher temperatures cause the acceleration of population growth and density peaks are achieved at a faster rate (PRIETO; CRUZ; MORALES, 2006). Moreover, temperature also affects the physiology of organisms, modifying fecundity, reproduction, as well as muscle activity, breathing, among others (CASTRO-MEJÍA et al., 2017).

The growth velocity (GV) parameter is closely linked to MDO, which reflects the organisms' capacity for proliferation and population growth. Production variables, such as growth, biomass, survival, and development time, provide measures of a population's relative success by representing the characteristics of their life history. Furthermore, these variables can serve as indicators of regulatory factors, such as temperature and food source quality (LEMKE; BENKE, 2004). In the present study, the higher GV values observed in *M. minuta* fed with *Chlorella* sp. compared to those fed with *S. cerevisiae* and fish broth suggests that this microalgae provides superior nutritional value.

The cultivation of planktonic organism in laboratory is important because it provides valuable information for large-scale production. Knowledge about the biology of species combined with laboratory observation enables the possibility of understanding how environmental factors and food type affect population growth. This allows adaptations of cultivation conditions to ensure successful production (SIPAÚBA-TAVARES; BACHION, 2002).

5 CONCLUSIONS

The analysis of the productive characteristics of *M. minuta* showed that the species exhibits high values for population growth variables, which highlights its suitability for large-scale production as a live food in aquaculture. Among the three treatments assessed, specimens fed with *Chlorella* sp. demonstrated the highest averages for the growth variables studied.
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