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Abstract. Population density in the culture of *Moina macrocopa* affects the production of offspring per parent. Female parthenogenesis is able to produce a high population density. Information on the *M. macrocopa* culture densities for inoculant provision is not yet available. This study aims to examine the density of the culture of *M. macrocopa* against the production of offspring per parent and the percentage of female parthenogenesis offspring production. The treatments in this study were differentiated parent densities ranging from 20 ind/ L to 330 ind /L and each with three replications. During the culture, *M. macrocopa* was fed with a rice bran suspension. The results of this study indicated that *M. macrocopa* was cultured using rice bran suspension feed in the parent density of 66 ind/L, which produced offspring per parent (8.56 to 13.01) equal to the parent density of 20 ind/L, whereas all of the females were parthenogenesis females. *M. macrocopa* was cultured with a mother density of 330 ind /L, which produced the highest female sexual ephippia and male offspring in the first until the third birthing round.

1. Introduction

M. macrocopa reproduces in both an asexual (parthenogenesis) and sexual way [1,2]. *Miona* sexual reproduction begins with female parthenogenesis producing male and female sexual offspring [3].Female sexual *Moina* are females that do not reproduce with parthenogenesis; instead, they reproduce through eggs from sexual females that will be fertilized by males and subsequently undergo a carapace thickening modification process to form ephippia [4]. The usage of sexual females as an inoculant will decline the population growth rate. Based on this, culture technology is needed to provide female parthenogenesis through culture to induce population density regulation.

Population density is a major factor that can influence fecundity and induce the production of male and female sexual *Moina* [3, 5). Offspring production per parent and the specific growth rate of *Moina* will decrease along with the population density increase [6]. *M. macrocopa* culture, with an initial density of 20 ind/ L fed using rice bran suspension feed (0.3 - 0.33 ml/ L), generated a high level of production of offspring per parent (13 ind parent)[7], and the offspring production was not different when the *M. macrocopa* was cultured using *Chlorella* spp feed (12-14 ind / parent) [8].

A *M. macrocopa* inoculants into mass culture is needed in large quantities and is usually obtained from high density cultures. Information on *M. macrocopa* culture densities for inoculant provision is not yet available. *M. macrocopa* culture in a high density can cause a decrease in offspring production and induces sexual reproduction [3, 5]. The inoculant quality is very influential on population growth and culture success. This aim of this study was to examine the density of the culture of *M. Macrocopa* against the production of offspring per parent and the percentage of female parthenogenesis offspring production.

2. Materials and methods

2.1 Research design

This research was conducted at the Nutritional Laboratory in the Department of Aquaculture in the Faculty of Fisheries of Marine Sciences, Institut Pertanian Bogor. This study was conducted using a completely randomized design (CRD) with different *M. macrocopa* density treatments. The *M. macrocopa* culture density in this study started from 20 ind /L as the initial density of *M. macrocopa* culture according to Delbare and Dhert [9], up to the parent density of 330/ L using a bran suspension feed which supported the *M. macrocopa* parthenogenesis reproduction. This was within the range of 22.2 mg/ L up to 24.42 mg/ L.

The treatment of the parent density in this study was Treatment 1. *M. macrocopa* parent density 20 ind/ L; Treatment 2. *M. macrocopa* parent density 66 ind/ L; Treatment 3. *M. macrocopa* parent density 132 ind/ L; Treatment 4. *M. macrocopa* parent density 198 ind/ L; Treatment 5. *M. macrocopa*parent density 264 ind / L and Treatment 6. Density of *M. macrocopa* parent 330 ind/ L.

2.2. Culture media

The media for the *M. macrocopa* culture in this study was water taken from the water tanks owned by IPB's Faculty of Fisheries and Marine Sciences. Water from the water tanks was used to fill a 1000 L fiber tank that was aerated at least three days before usage. Water from the fiber tank was filtered with 40 µm nylon before it was put into an experimental tank to eliminate other competing zooplankton.

2.3. Rice bran suspension production

Rice bran suspension was made by suspending as much as 100 g of rice bran into 500 ml of water, using a blender at a constant speed of 2000 rpm for 5 minutes two times. The second suspension is performed after 30 minutes of the first suspension. The water suspension was filtered using 2 mm, 0.1 mm and 40 µm sieves. The suspensions that passed through the filtration then had more water added to reach a volume of 500 ml. The results of the proximate analysis of the rice bran suspension contained of dissolved organic matter as follows: 72 mg/ mL, protein 0.83%, and fat 0.79%.

2.4. Provision of inoculants and M. macrocopa culture

The *M. macrocopa* used in this study had been adapted to a bran suspension feed for at least 2 months in a culture of 10 L of water with a density of 20ind/ L. The cultured offspring (aged less than 24 hours) became the inoculant (of this study) with a density of 20 / L to 330 / L in a 300 mL water volume. This *M. macrocopa* culture used a bran suspension feed which had the same provision as the offspring for inoculants, which was 0.3 ml / L on the first day and 0.33 ml / L on the second to fourth day.

M. macrocopa was cultured with a density of 20-330 ind / L using aeration at a speed of 28 ml/ minute. The dissolved oxygen in the first study was 4.0 mg/ L and after 20 hours of culture, it was ranged from 3.6 to 3.0 mg/ L. The water hardness was 53.09 mg / L pH 7.8-7.7. The water temperature was 27-30 ° C and total amount of ammonia was less than 0.25 mg / L (Table 1).

Table 1. Water quality in *M. macrocopa* culture

Water Quality	1st day	2nd day	3rd day	4th day
Dissolved Oxygen (mg/L)	3.33 - 3.70	3.13 - 3.67	3.03 - 3.60	3.07 -3.57
PH	7.8 -7.7	7.8 -7.7	7.7	7.7
Ammonia (mg/L (NH3/NH4)	0 - 0.25	0 - 0.25	0 - 0.25	0 - 0.25
Temperature (°C)	27 - 30	27 – 30	27 -30	27 – 30
Water Hardness	53.09	53.09	53.09	53.09

M. macrocopa culturing was carried out for 5 days in a laboratory with daytime lighting, ranging from 700-900 lux and with the range in the evenings being 50 to 100 lux. On the second, third and fourth days, they were harvested and the *M. macrocopa* offspring were separated from their mothers by filtering. The *M. macrocopa* offspring were then cultured with a 1000ind/L density [10] with the addition of a 36 ml/minute aeration with daily water changes. During the rearing period, the *M. macrocopa* offspring were fed with a rice bran suspension, with a range of 37 mg/ L on the first day, 49 mg/ L on the second day, 58.5 mg/ L on the third day, and 70 mg/ L on the fourth and fifth days.

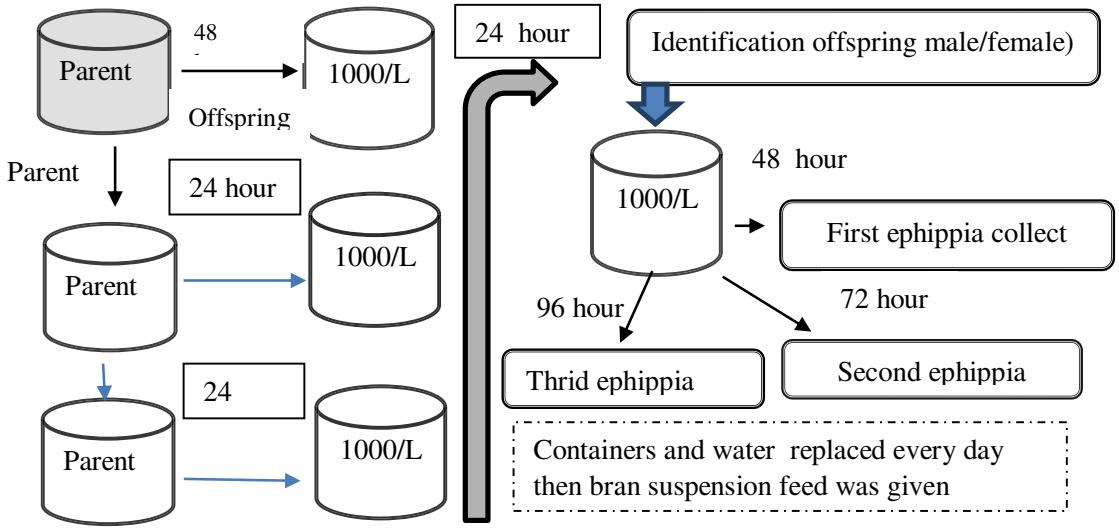


Figure 1. Workflow of the *M. macrocopa* preservation in the relation to the male offspring production test and ephippia

2.5. Observation result

The *M. macrocopa* offspring resulting from the harvest on the second, third and fourth days after being cultured for 24 hours were then calculated. The identification of their sex then was made on the second day using binocular microscopes with a 50 x magnification. The data was used to calculate the production of offspring per parent and the percentage of male offspring based on the equation below.

$$\text{Offspring Production per Parent} = \frac{\text{Total offsping } M.macrocopa}{\text{Total parent } M.macrocopa}$$

$$Male\ Percentage = \frac{\text{Total male } M.macrocopa \text{ Offspring}}{\text{Total } M.macrocopa \text{ offsping}}\ x\ 100\%$$

After the offspring were re-cultured for two days, the ephippia was taken until the fifth day with a pipette, and then put in a petri dish. The next step was to rinse and transfer it into a new petri dish before being washed three times. Furthermore, the amount of ephippia was counted. The data was then used to calculate the percentage of sexual females/ephippia against the total number of females using the equation below.

$$Ephipia\ from\ the\ total\ female\ (\%) = \frac{\text{Ephippia production}}{\text{Total female}}\ x\ 100$$

During the culture period, the water quality measurement was made, covering dissolved oxygen, pH, temperature, hardness and total ammonia.

2.6. Data analysis

The observation data was analyzed using ANOVA. If from the results from the analysis of variance showed that the treatment had significantly different results, then it was followed by Duncan's Multiple Range Test in order to determine the treatment with the best response at a 95% trust degree.

3. Results and discussion

3.1. Result

The *M. macrocopa* culture with a parent density of 20-303 ind/ L had a high level of life (100% - 92.33%) (Figure 2). The production of offspring per mother in the *M. macrocopa* in culture with 20-330 ind/ L from the first birth was between 6.08 - 8.85 ind/ parent, and from the second birth, this increased to 7.85 - 13.37 ind/ parent. The third birth was 6.02 - 12.73 ind / parent. The *M. macrocopa* culture with a parent density of 66 ind/ L and 20ind/ L produced the highest number of offspring per parent in the first birth, compared to the second and third (Figure 3).

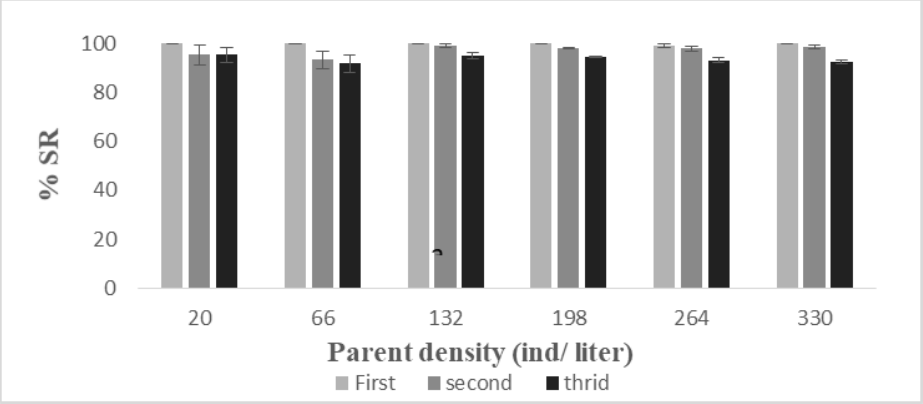


Figure 2. Survival rate of the *M. macrocopa* parents after the first, second and third births in culture with different parent densities.

The *M. macrocopa* culture with the highest parent density (264 ind/ L and 330ind/ L) resulted in the lowest production of offspring per parent in the first birth, up until third birth which was 6.17 - 9.19 and 6.08 - 7.85. The *M. macrocopa* culture with a parent density of 330 ind/ L produced the highest percentage of males at 0.27% in the first birth and this increased to 0.42% in the second birth and 0.52% in the third birth. The culture of *M. macrocopa* with a parent density of 264 ind/ L resulted in the production of male offspring in the second birth at 0.09% and this increased to 0.12% in the third birth (Figure 4A).

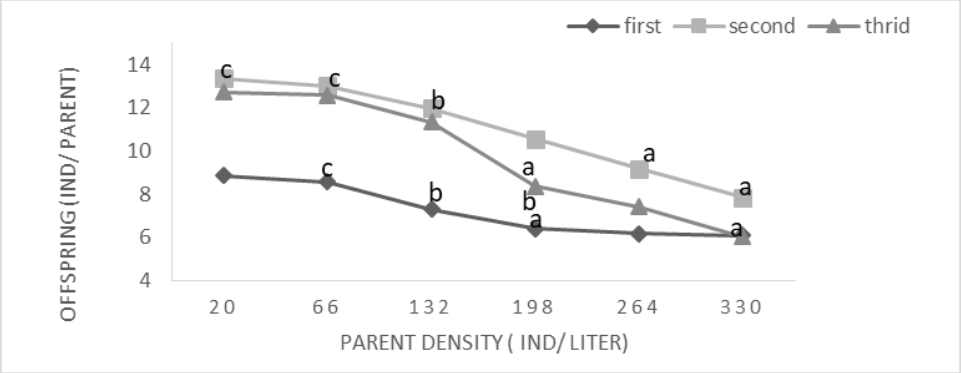
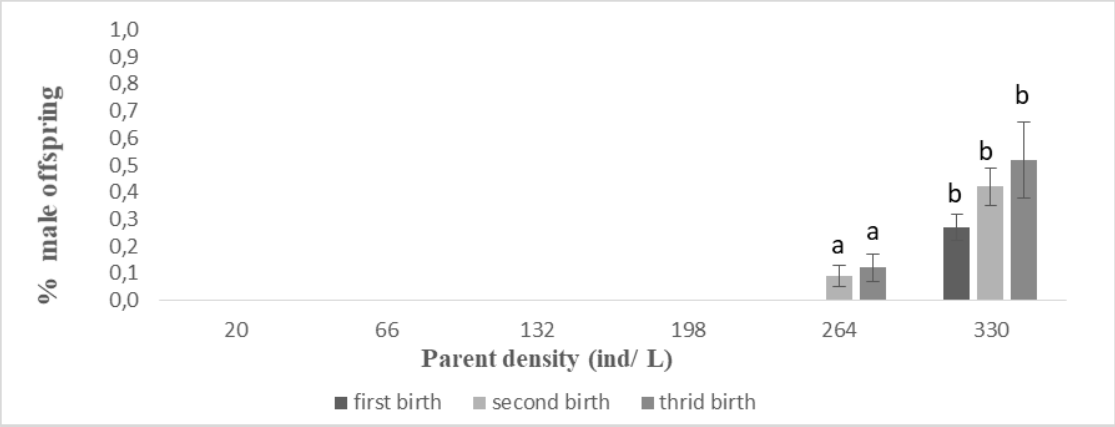


Figure 3. Production of offspring per mother of first, second and third birth from cultured *M. macrocopa*with different parent densities.
Remarks: Different small letters on the same sampling day is indicating significant differences (P <0.05)

The results from culture of female offspring of *M. macrocopa* with a density of 1000 ind/ L fed with a rice bran suspension feed was 37 -70 mg/ L for the first to fifth days, producing ephippia from the culture of offspring with a parent density of 132 - 330 ind/ L. The highest production of ephippia *M. macrocopa* resulted from cultured female offspring with a parent density of 330 ind/ L at 5.08% in the first birth, 4.98% in the second birth and 3.78% in the third birth (Figure 4B).

A



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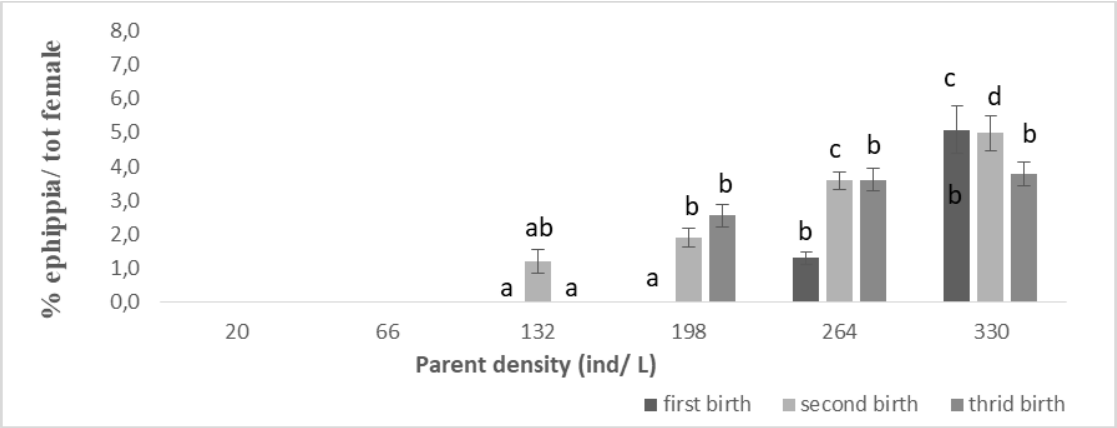

Figure 4. Production of male offspring (A) and the percentage of ephippia to the total female (B) *M. macrocopa* with different density cultures.
Remarks: Different small letters on the same sampling day indicates significant differences (P <0.05)

The culture of *M. macrocopa* with a parent density of 20 ind/ L and 66 ind/ L did not produce male and female offspring that produced ephippia, but the population instead produced parthenoogenesis females in the first, second and third births. The culture of *M. macrocopa* with a density of 66 ind/ L was the maximum density required to produce one hundred percent female parthenogenesis.

3.2. Discussion
The quality and quantity of the food is one of the important factors in *M. macrocopa* culture that directly affects population growth [11]. Increased parent density in *M. macrocopa* culture was linked to the increase in the total production of the offspring, but reduced the offspring production per parent. The increased in the parent density caused a decrease in the concentration of food per parent, which caused a decrease in the production of offspring per parent and the specific growth rate of *M. macrocopa* [6]. The culture of *M. macrocopa* used a rice bran suspension feed with a parent density of 66 ind / L which resulted in the production of offspring per parent (13.01 ind/parent). This was the same as the parent density of 20 ind \/ L (13.37 ind/parent).

The production of offspring per *M. macrocopa* mother cultured using the rice bran suspension feed with a parent density of 66 ind/ L was no different to the *M. macrocopa* which had been cultured using the *Chlorella spp* feed (12-14 ind/ parent) [8]. The nutritional quality of the rice bran suspension was 11.16% protein and 10.62% fat supporting *M. macrocopa* reproduction. The *M. macrocopa* culture using rice bran suspension feed with a parent density of 20 ind/ L and 66 ind/ L did not produce male and female sex offspring from the first to the third birth. *M. macrocopa* culture with a parent density of 66 ind/ L is the maximum parent density that produced 100% parthenogeneous females offspring.

M. macrocopa culture was done using different initial inoculants, ranging from 20 ind/ L density [9], 25 ind/ L (12 and 50 ind / L [12, 13]. Based on the results of this study, the initial inoculant was still optimal to increase *M. macrocopa's* initial growth. The population growth in the *M. macrocopa* culture is not only determined by the density of inoculants but also by the female species of *M. macrocopa*, namely the availability of female *M. macrocopa* parthenogensis.

Offspring production per parent and the specific growth rate of *M. macrocopa* will decrease with the increase in population density and with the decreasing feed concentration [6]. The culture of *M. macrocopa* with a parent density of 264 ind/ L and 330 ind/ L produced the lowest amount of offspring per parent and produced more male offspring as well. According to Smirnov [14], the formation of male offspring was influenced by environmental factors including population density, temperature, nutrition and the accumulation of metabolic products. The quality and quantity of the food and population density are the main factors that can induce male production and ephippia in *D. magna* [15].

The production of male cultured *M. macrocopa* with a parent density of 330 ind/ L was low due to the low population density and high feed concentration. The high concentration of arginine and histidine in the rice bran triggered the production of parthenogenic female offspring and decreased the production of male offspring [16, 17, 18]. The changing of the culture media every day may eliminate the influence of the metabolic products which became a factor involved n the production of male *Moina*.

A high population density with sufficient feed conditions can induce sexual female production [5]. Ephippia *M. macrocopa* are produced by newly born female offspring and do not reproduce through parthenogenesis [4]. Moina's gametogenesis process to produce ephippia eggs only occurs when populations have a high density with sufficient feed [19]. On the contrary, the availability of excess feed can increase the size of the females in the first reproduction, increasing the rate of somatic growth and fecundity [6.19].

The production of ephippia *M. branchiate* was 53% from a culture of offspring resulting from a culture with a mother density of 750 ind/ L [6]. Moina females from high-density parent cultures that are maintained at high densities have the potential to produce higher ephippia [6, 20].

4. Conclusion

The culture of *M. macrocopa* using a rice bran suspension feed with a parent density of 66 ind/ L produces offspring per parent at 8.56 to 13.01 ind, which is the same as the parent density of 20 ind/ L, where all of them are parthenogenesis females. The culture of *M. macrocopa* with a parent density of 330 ind/ L produces female sexual/ephippia and male offspring from the first until the third birth.

5. References

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