ABSTRACT

*Mo*ina *macro*copa* is a natural feed that can be cultured to producing *ephippia* as a bioproduct for fish and shrimp larvae feed. The number of males in the population affects the quality and quantity of *ephippia* produced. This study was conducted with the purpose of examining the female age in *M. macrocopa* mating and examining the ratio male-female *M. macrocopa* in the mating on the quantity and quality of *ephippia* produced. The treatment in this research was the ratio male-female sex of 1:30, 3:30, 5:30, 7:30, 9:30, 12:30, 15:30. Male and female sexual offspring *M. macrocopa* were produced from cultured using a combination of induction factors such as density, feed concentration, kairomones and dissolved oxygen. Male and female offspring produced were cultured with a density of 1000 ind/L. This mating culture was using a container with a volume of water of 2 ml per individual. The results of this study indicated that mating *M. macrocopa* using 70-hour old sexual females resulted in the highest *ephippia* production. Mating *M. macrocopa* with a sex ratio of 9:30 (male and female sexual) were resulted in *ephippia* containing two eggs of 100%, with *ephippia* hatching degree of 35.4–38.3%.

**Key words:** culture, hatching, *ephippia*, eggs, *rice bran*
INTRODUCTION
The rising price of Artemia cysts makes Moina one of the natural food choices for fish and shrimp larvae, due to its body size (± 400 um) and nutritional value comparable to Artemia. Ephippia M. macrocopa can be stored for up to 70 years and hatched at any time in a relatively short time (18). The production of ephippia M. macrocopa is still constrained in terms of both quantity and quality of ephippia, which is currently produced has low hatchability. One effort to increase the quantity and quality of ephippia is by regulating the sex ratio of male females in the mating of M. macrocopa. Male and female sex ratios in Moina mating affect the success of conception, the number of ephippia containing an embryo and the number of ephippia containing two embryos. Ephippia M. macrocopa only produced by female sexual offspring who do not undergo reproductive parthenogenesis (2). Production of male offspring and ephippia cladosera can be induced by the quality and quantity of feed (10), population density (22), temperature, water quality and fish kairomone. Our previous results showed that male and female sexual M. macrocopa can be produced from culture by combining several induction factors simultaneously including density, the concentration of ricebrain suspension and carp feces (kairomone) (15). Culture of M. Macrocopa without aeration with a parent density of 660 ind/L using ricebrain suspension of 45-54 µg/parent resulted in the production of M. macrocopa male offspring (253-263 ind/L). M. macrocopa culture used aeration with a parent density of 660 ind/L, ricebrain suspension of 56-80 µg/parent, goldfish feces 3.86 mg/L was produced the females with the highest ephippia production (6069 grains/L). Conde et al. (5) stated that if the egg cells (resting eggs) from the sexual female M. macrocopa are not fertilized by the males than it will be described as ephippia without eggs or (the eggs is) damaged. The quantity and quality of males affected the amount of ephippium fertilized in ephippia or the number of ephippia containing two eggs (4). Highly number male in population will increases the number of sexual females being married (21). According to Leung, (12), he reported that the sex ratio of male and female M. australiensis in mating is 4:5. With this research, an optimal ratio of sexual males and females will be obtained in the mating of M. macrocopa which is useful for increasing the quantity and quality of M. macrocopa ephippia that being produced. This study aims to examine the sex ratio of male and female age on the quantity and quality of ephippia M. macrocopa produced.

MATERIALS AND METHODS
Research design
This study uses a completely randomized design (CRD), which consists of two sub-studies. First are the effective age of female sexual offspring induced in M. macrocopa mating and the second is the sex ratio of males and sexual females which is as follows: A). 1:30, B). 3:30, C). 5:30, D). 7:30 E). 9:30, F). 12:30 and G). 15:30. Production of the male and sexual female offspring M. macrocopa. M. macrocopa sexual females were produced from a culture with a parent density of 660 ind/L using aeration (28 ml/min), ricebrain 56-80 µg/parent suspension, 3.86 mg/L goldfish feces. M. macrocopa male offspring were produced from a culture with a parent density of 660 ind/L and using a ricebrain-45-54 µg/parent suspension. The males and females are obtained on the third day and separated from the mother by screening. Offspring was cultured with a density of 1000 ind/L with ricebrain suspension feed that supports the production of ephippia (22). During the rearing period, M. macrocopa fed with ricebrain suspension concentrations, as in Table 1. The cultured M. macrocopa was conducted in a confined space with daytime lighting of 700-900 lux and 50 -100 lux nights. On the second day (24-hour age) identification and separation of males, especially in the culture for the provision of sexual females. Male are kept until 3 days (72 hours) before being used in mating culture.
Table 1. Ricebran suspension concentrations for induction of production of males, sexual females and the culture of *M. macrocopa* offspring

| Day | Ricebran Suspension (mg/L) |
|-----|---------------------------|
|     | Induction of males        | Induction of ephippia | Rearing offspring |
| 1   | 29.60                     | 37.00                  | 37.00             |
| 2   | 32.64                     | 44.88                  | 44.88             |
| 3   | 36.86                     | 53.85                  | 53.85             |
| 4   | 40.39                     | 53.85                  | 64.32             |

Ricebran suspension made using an amount of 100 grams of the ricebran was suspended in 500 ml of water using a blender at a speed of 2000 rpm for 5 minutes twice. The second suspension was done 30 minutes after the first suspension. Then the suspensions were filtered using 2 mm, 0.1 mm and 40 µm filters. The suspensions that passed the filtration then had more water added to reach a volume of 500 ml. The results of proximate analysis of the ricebran suspension contained 74 mg/ml organic materials, 0.83% protein, and 0.79% fat (14).

**Determination of the age females in *M. macrocopa* mating**

Female offspring were isolated from the cultivation stock of *M. macrocopa* as a result of the sexual induction of females at the age of 0 hours, 24 hours, 48 hours and 70 hours. Then the female child is placed in a mating container with a diameter of 3 cm, each containing 30 female *M. macrocopa* in 60 ml of water (22). Every day 100% of the water is replaced and the container replaced. Simultaneously with water replacing the addition of ricebran suspension feed for the maintenance of offspring under the age of *M. macrocopa*, as shown in Table 1. Ephippia can be collected and counted on the fourth to the sixth day of each treatment. Ephippia were further identified using a binocular microscope with a magnification of 100x to determine the number of eggs in ephippia. The percentage of ephippia to total females and the percentage of ephippia containing x eggs (x is the number of eggs in ephippia) was calculated using the equation below;

\[
\% \text{ ephippia contains (x) eggs} = \frac{\text{amount ephippia berisi (x) telur}}{\text{Jumlah total ephipia}} \times 100\%
\]

\[
\% \text{ ephippia per total female} = \frac{\text{Jumlah ephippia moina}}{\text{Jumlah induk betina moina}} \times 100\%
\]

During the culture period, water quality measurements were carried out which included dissolved oxygen, pH, temperature and total ammonia.

**Test of ephippia hatchability**: Collection and counting of ephippia *M. macrocopa* was done from day five to day six eggs. The ephippia were identified based on containing eggs using a binocular microscope (100x magnification). The percentage of ephippia from the total population and the percentage of ephippia...
containing two, one, and no eggs were calculated using the following equation:

\[
\text{Ephippia from the total population (\%)} = \frac{\text{Ephippia production}}{\text{Population Moina}} \times 100
\]

\[
\text{Ephippia containing (n) eggs (\%)} = \frac{\text{Number of ephippia containing (n) eggs}}{\text{Total ephippia production}} \times 100
\]

Note: n is the number of eggs in the ephippia

During the culturing period, the water quality was assessed for its dissolved oxygen, pH, temperature and total ammonia and hardness. Ephippia hatching rate assessment using ephippia \textit{M. macrocopa} containing two eggs was stored wet in a microtube containing of distilled water at a density of 200 eggs/ml. Ephippia from each treatment after being stored for two months, incubated in a glass container containing 300 ml of water with a lighting intensity of 1800 lux (10). The hatching \textit{M. macrocopa} was removed and counted on the second and third days. The degree of hatching ephippia was calculated based on the equation in Haghparast \textit{et al.} (12) where \(I_i\) is the hatching index and \(N_i\) is the number of larvae that hatched.

\[
\text{Hatching rate} = \sum_{i=3}^{15} \frac{N_i}{N_e} \times I_i
\]

Data Analysis

The observational data did not show good homogeneity so that Kruskal Wallace was tested (nonparametric) to determine the treatment with the best response at a 95% confidence level.

\section*{RESULTS AND DISCUSSION}

Age and percentage of sexual females/ephippia

Nurture of female offspring of 70-hour-old female stock in a marital container produces the highest percentage of ephippia production in the total female population of 89\%. Nurture of female directly induced into the mating container produces females with a percentage of ephippia production of a total female of 2\% (Figure 1.). Ephippia is produced by \textit{M. macrocopa} at the age of four days to six days with the most production at the age of five days.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Percentage of ephippia to the total female \textit{M. macrocopa} in the care of female offspring in a mating container with ages 0, 24, 48, 70 hours. Note, different lowercase letters on the same \textit{M. macrocopa} ages, show a marked difference (P <0.05) }
\end{figure}

Sexual female \textit{M. macrocopa} has been identified as producing a sexual egg that is characterized by a change in the ovary to darkness at the age of 75-80 hours. Female Moina was seen to have two eggs in a large, dark at 80 hours (Figure 2B and Figure 2C).

Male \textit{M. macrocopa} can be identified from the size of the first antenna which is longer (Fig. 2A). \textit{M. macrocopa} male which is three days old, has a body size of 1.0 - 1.1 X 0.8 – 0.5 mm, about half the size of a sexual female (1.75 – 2.0 x 1.0 - 1.3 mm).
Sex ratio in M. macrocopa mating

The number of males in mating does not affect the number of sexual females that produce eggs but does affect the number of fertilized ephippia (Fig. 3A). The mating of M. macrocopa using male and female sex ratios of less than 7:30 results in ephippia containing two eggs of less than 60%. The mating of M. macrocopa with a male and female sex ratio of more than 9:30 produces ephippia containing two eggs at 100% (Fig. 3B).

Figure 3. Percentage of ephippia to total females (A) and ephippia containing two eggs (B) in the mating of M. macrocopa using different female sex ratios from female offspring aged 70 hours. Note, different superscript on ephippia production, showed the real difference (P<0.05).
Male and female sex ratios in *M. macrocopa* mating affect the degree of hatching of ephippia being produced. The degree of hatching of ephippia from a mating using a 70-hour-old female at the mating ratio of 9:30, 12:30 and 15:30 was not significantly different by 36.17%, 38.26% and 36.67% respectively. The degree of hatching of the lowest ephippia came from matings with a 1:30 sex ratio of 9.92% (Figure 4). Water quality during the study which included temperatures ranging from 28-30 °C, water pH 7.5-8, with hardness of 55 mg/L. Dissolved oxygen at the beginning of the study was 4 mg/L and decreased at the end of the study to 2.5 mg/L. Total ammonia after 24 hours of maintenance is less than 0.250 mg/L as shown in Table 3.

![Figure 4. Percentage of hatchability of ephippia from the mating of *M. macrocopa* using different female sex ratio from female offspring aged 70 hours. Note, different superscript on ephippia, hatchability, showed the real difference (P<0.05).](image)

### Table 3. Results of measurements of water quality parameters in the mating of *M. macrocopa*

| No | Parameter            | Value       |
|----|----------------------|-------------|
| 1  | Temperature          | 28 – 30°C   |
| 2  | Ph                   | 7.5 – 8     |
| 3  | Hardness             | 55 mg/L     |
| 4  | Dissolved Oxygen     | 4 – 2.5 mg/L|
| 5  | Total Amount of Amoniac | 0.00 – 250 mg/L |

Female offspring of *M. macrocopa* induced by sexual females remain as sexual females and producing ephippia while maintained at low densities (30 ind/60 ml) when they are 70 hours old. Our observations also show that the sexual female *M. macrocopa* begins to synthesize its sexual eggs at 75-80 hours of age. *M. macrocopa* cannot transition from asexual reproduction into direct sexual reproduction, but female parthenogenesis *M. macrocopa* can produce sexual females and males from the development of eggs parthenogenesis to producing ephippia (6). Female child *M. macrocopa* becomes sexually reproductive when it fails to reproduce the first parthenogenesis (2). *M. macrocopa* used in this study was first given birth at 65 hours. Efforts to increase the production of sexual females can be made by maintaining *M. macrocopa* broodstock by regulating feed concentration and density and using kairomone (15). Furthermore, the female child was raised with high density (1000 ind/L) (22) with the regulation of feed concentration.. The results of this study indicate that *M. macrocopa* females transitioned as sexual females at around 70 hours of age so that they can be used for marital testing. Moina mating consists of three phases beginning with the male capturing sexual females, then followed by male movements to position themselves perpendicular to sexual females, then copulation lasts for 16-25 seconds (8). The behavior of male *M. macrocopa* in mating.
differs from Daphnia, which only attacks sexual females in the condition of synthesizing eggs in the ovaries and has not been released in the incubation chamber (9). The duration of mating for daphnia and Moina lasts for 8-10 minutes (9) The results of this study show that the number of males in mating does not affect the number of sexual females that produce ephippia, but does affect the number of perfectly fertilized ephippia (containing two eggs) (Figure 5). Conde et al. (5) state that, egg cells (ephippium) from M. macrocopa sexual females, if eggs are not fertilized, will be released as ephippia without eggs or damaged. Mating M. macrocopa with a low male sex ratio produces ephippia without eggs or high damaged eggs. Mating of M. macrocopa with male and female sex ratio 9:30 - 15:30 produces ephippia containing two eggs at ± 100%. The sex ratio of male and female sexual M. macrocopa 9:30 is the lowest mating sex ratio that produces ephippia containing two eggs at 100%. According to Leung (12), the sex ratio of male-female M. australiensis is 4: 5. In our study, the sexual females used began to produce ephippia at the age of four days (96 hours) to six days. The production of ephippia that is not simultaneous causes the ratio of male and female sexual in this study is lower than the results of research Leung (120 on M. australiensis. The quality and quantity of feed affect the ability of male sexual activity, among others, the length of copulation so that it affects the probability of fertilization is higher (4). Male and female sex ratios in M. macrocopa mating affected the degree of hatching of ephippia produced. In matings with female sex ratios of 9:30, 12:30 and 15:30 produce no different degree of hatching (36,17-38,26%). The hatchability of Moina's ephippia is influenced by several factors, including irradiation, temperature, pH, and duration of storage (Stross, 1966). Light intensity and pigment concentration in ephippia skin can cause variations in the value of hatching degrees, where pigments in ephippia skin function to protect the embryo against UV radiation, so a decrease in pigment concentration during storage will increase sensitivity to light at the time of hatching (16 ). In this study, M macrocopa was given ricebran suspension feed. The low hatchability of ephippia M. macrocopa with bran suspension feed is due to the low content of EPA fatty acids and α-linolenic acid (0,20 – 0,27%) in ricebran (7). The concentration of EPA in feed affects the production of ephippia (1) and also the degree of hatching (4 , 19). Some species of cladoceran are reported to be able to convert α-linolenic acid to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with varying abilities between species (13). Bran also contains high linoleic acid (6,35 – 6,85%). Linoleic acid can be converted to arachidonic acid and docosapentaenoic acid which can be oxidized to DHA (3). The results of the research of the female-male sex ratio in M macrocopa mating will encourage further research, especially food engineering in order to improve the quality and quantity of ephippia Moina. The availability of Ephippia Moina will improve business sustainability in aquaculture.

**Conclusion**

The results of this study were indicated that mating M. macrocopa using 70-hour old sexual females resulted in the highest ephippia production. The mating of M. macrocopa with sex ratio male-female of 9:30 (male and female sexual) have resulted of ephippia containing two eggs of 100%, with ephippia hatching degree of 35,4- 38,3%.

**REFERENCES**

1. Abrusan. G., P. Fink and W., Lampert . 2007. Biochemical limitation of resting egg production in daphnia. Limnology Oceanography, 52, 1724–1728
2. Alekseev V., B. Stasio and J. Gilbert.. 2007. Diapause in aquatic invertebrates: theory and human use. Springer Science and Business Media
3. Chang M. I., M. Puder K.M., Gura K.M. 2012. Review, The use of fish oil lipid emulsion in the treatment of Intestinal Failure Associated Liver Disease (IFALD). Nutrients, 4, 1828-1850
4. Choi J., Kim S., G. Hwan La, K., Chang D., Kim ,K. Jeon . M., Park , J. Joo , H. Kim and K. Jeong .. 2016. Effects of algal food quality on sexual reproduction of Daphnia magna. Ecology dan Evolution. John Wiley and Sons Ltd
5. Conde-Porcuna J., P. Valdés ,S. Romo and C. Pérez-Martínez .2011. Ephippial and
substantaneous egg abortion: relevance for an obligate parthenogenetic Daphnia population. Journal Limnology, 70, 69-75
6. Dodson S., C. Caceres and C. Rogers. 2010. Ecology and classification of north american freshwater invertebrates. chapter 20. Cladocera and other Branchiopoda. Academic Press
7. Faria S., P. Bassinello and M. dan Penteado. 2012. Nutritional composition of ricebran submitted to different stabilization procedures. Brazilian Journal of Pharmaceutical Sciences, 48
8. Forro L. 1997. Mating behavior in Moina brachiata (Crustacea: Anomopoda). Arch. Hydrobiol. 360
9. Freedberg S., and D. R. Taylor. 2007. Sex ratio variance and the maintenance of environmental sex determination. Journal Evolutionary Biology, 20, 213-220
10. Haghparast S.,A. Shabani . B., Shabanpour and S., dan Hoseini. 2012. Hatching requirements of Daphnia magna Straus, 1820, dan Daphnia pulex Linnaeus, 1758, diapausing eggs from Iranian populations in vitro. Journal Agriculture. Science Technology, 14, 811-820
11. Jaime Y.F. 2009. Reproduction of the zooplankton, Daphnia carinata dan Moina australiensis: Implications as live food for aquaculture dan utilization of nutrient loads in effluent. Thesis School of Agriculture Food dan Wine, The University of Adelaide
12. Leung F.J. 2009 Reproduction of the zooplankton, Daphnia carinata and Moina australiensis: Implications as live food for aquaculture and utilization of nutrient loads in effluent. Thesis from School of Agriculture, Food and Wine, The University of Adelaide
13. Masclaux H., A. Bec . M. K., Kainz . F., Perrie , C. Desvilettes and G. Bourdier. 2012. Accumulation of polyunsaturated fatty acids by cladocerans: effects of taxonomy, temperature, and food. Freshwater Biology 57, 696–703
14. Mubarak, A. S., D. Jusadi, M.Jr. Zairin. M. Jr., and M.A.Suprayudi. 2017a. Evaluation of the rice bran and cassava suspension use in the production of male Moina macrocropa offspring and ephippia. AACL Bioflux, 10, Issue 3
15. Mubarak, A. S, D. Jusadi., M. Z. Junior and MA Suprayudi. 2017. Production of Ephippia Moina macrocropa with Feed Manipulation, Density, "Kairomon" Fish and Oxygen Solubility. [Dissertation]. Graduate School. Bogor Agricultural Institute. Bogor
16. Pinceel T, B. Vanschoenwinkel, J. Uten and B. Brendonck . 2013. Mechanistic and evolutionary aspects of light-induced dormancy termination in a temporary pond crustacean. Freshwater Science, 32, 517–524
17. Putman A., D. Martin-Creuzburg , B. Panis and L. De Meester . 2015. A comparative analysis of the fatty acid composition of sexual and its plasticity as a function of food quality. Journal Plankton Research, 37: 752 – 63
18. Radzikowski J. 2013. Resistance of dormant stages of planktonic invertebrates to adverse environmental conditions. Journal Plankton Research, 35,707-723
19. Sperfeld E.and A. Wacker . 2011. Temperature affects limitation of D magna by eicosapentaenoic acid and the fatty composition of body tissue dan eggs. Freshwater Biology, 57, 497–508
20. Stross, R.G., 1966. Light and temperature requirements for diapause development and release in Daphnia. Ecology, 47, 368 -374
21. Winsor G. dan and D. Innes . 2002. Sexual reproduction in Daphnia pulex (Crustacea: Cladocera): observations on male mating behavior dan avoidance of inbreeding. Freshwater Biology, 47, 441-450
22. Zadereev E. and T. Lopatina . 2007. The induction of diapause in Moina by species-specific chemical cues. Aquaculture Ecology, 41, 255–261.