OPTIMAL RATIO OF MALE AND SEXUAL FEMALE IN THE MATING OF *MOINA MACROCOPA* (CRUSTACEA, MONIDAE)

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ABSTRACT

***Moina macrocopa* 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 were 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) ware resulted in ephippia containing two eggs of 100%, with ephippia hatching degree of 35.4- 38.3%.**

Keyword : culture, hatching, ephippia, eggs, ricebran

النسبة المثلى للذكور والإناث في تزاوج مونا ماكروكوبا (قشريات ، مونيداي)

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المستخلص

Moina macrocopa **هو علف طبيعي يمكن استزراعه لإنتاج نبات الإفيبيا كمنتج حيوي لتغذية يرقات الأسماك والروبيان. يؤثر عدد الذكور في السكان على جودة وكمية الإفيبيا المنتجة. أجريت هذه الدراسة بهدف فحص عمر الأنثى في تزاوج *M. macrocopa* وفحص نسبة الذكور إلى الإناث *M. macrocopa* في التزاوج على كمية ونوعية *ephippia* المنتج. كان العلاج في هذا البحث هو نسبة الجنس بين الذكور والإناث من 1:30 ، 3:30 ، 5:30 ، 7: 30. 9:30. ، 12:30 ، 15:30. ينتج من المستنبتات باستخدام مجموعة من العوامل البحثية مثل الكثافة وتركيز العلف والكيروموناتوالأكسجين المذاب. تم استزراع ذكور وإناث ذرية بكثافة 1000 إندون / لتر. تم استخدام وعاء به ماء بحجم 2 مل لكل فرد. أشارت نتائج هذه الدراسة إلى أن تزاوج *M. macrocopa* باستخدام إناث جنسية بعمر 70 ساعة أدى إلى أعلى إنتاج للإفيبيا. تزاوج *M. macrocopa* بنسبة جنس 9:30 (ذكور وإناث) نتج عنه احتواء الافيبيا على بيضتين بنسبة 100؟ ، ودرجة فقس الافيبيا 35.4 – 38.3؟.**

الكلمة المفتاحية : الثقافة ، الفقس ، الافيبيا ، البيض ، نخالة الأرز

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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 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 ricebran suspension and carp feces (kairomone) (15). Cultutre 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). Higly 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), ricebran 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 ricebran-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 ricebran suspension feed that supports the production of ephippia (22). During the rearing period, *M. macrocopa* fed with ricebran 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% proteine, 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 replacements the addition of ricebran suspension feed for the maintenance of offspring under the age of *M. macrocopa*, as shown in Table 1. Ephippia collected and counted on the fourth to the sixth day of each treatment of the female age.

Mating of *M. macrocopa*

The mating of *M. macrocopa* used 30 female offspring each with 30 ml/ind water volumes (21 , 22). The sex ratio of male and female sexual in this study was: A). 1:30, B). 3:30, C). 5:30, D). 7: 30 E). 9:30. F). 12:30 and G). 15:30. This mating uses M. macrocopa which was taken from the stock cultrure of the sexual females of a certain age, which results in high ephippia production after being transferred to the marting container based on the previous research stage. During mating, 100% of the water was 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;

$$\%\ \text{ephippia contains (x) eggs} = \frac{\text{amaunt ephipia berisi (x) telur}}{\text{Jumlah total ephipia}} \text{ X100\%}$$

$$\%\ \text{ephippia per total female} = \frac{\text{Jumlah ephipia moina}}{\text{Jumlah induk betina moina}} \text{ X 100\%}$$

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 base 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:

$$ephipia\ from\ the\ total\ population\ (\%) = \frac{\text{Ephippia production}}{\text{Population Moina}}\ x\ 100$$

$$ephipia\ containing\ (n)\ eggs\ (\%) = \frac{\text{Number of ephippia containing (n)}\ eggs}{\text{Total ephippia production}}\ x\ 100$$

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 *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 *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 *et al.* (12) where Ii is the hatching index and Ni is the number of larvae that hatched.

$$Hatching\ rate = \sum_{i-3}^{15} \frac{N\ i}{N\ e}\ x\ I\ i$$

$$= \sum_{i=3}^{15} \frac{N_i}{N_e} \times I_i$$

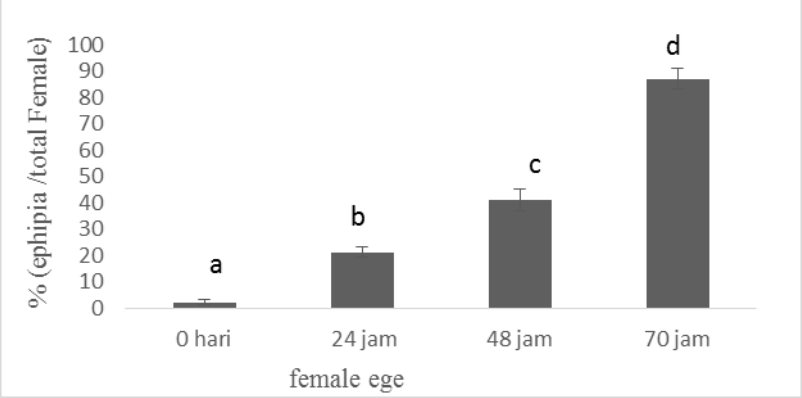
Data Analysis

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.

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 *M. macrocopa* at the age of four days to six days with the most production at the age of five days.



Picture 1. Percentage of ephippia to the total female 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 M. macrocopa ages, show a marked difference (P <0.05)

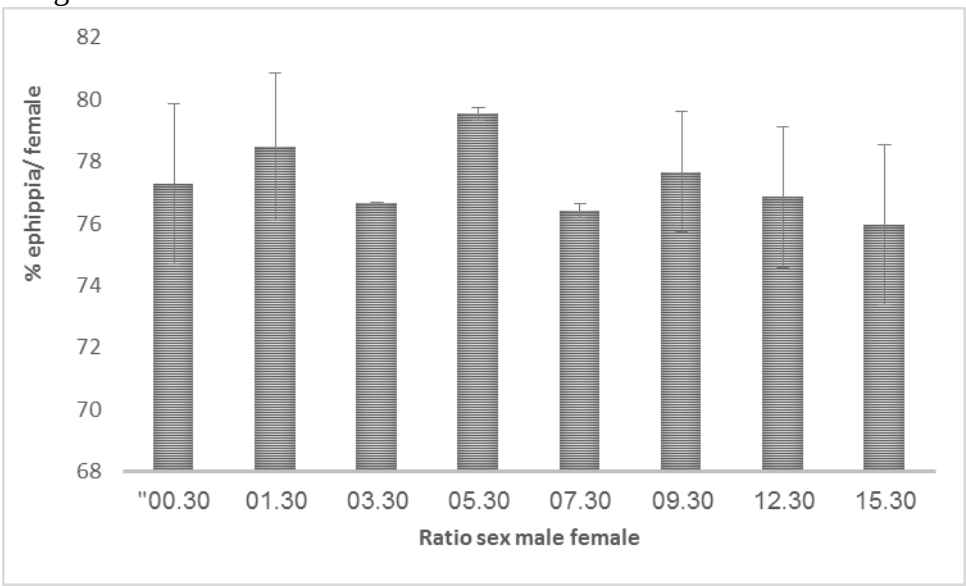
Sexual female *M. macrocopa* has been identified as producing a sexual egg that is characterized by a change in the ovary to darkness at the age of 75-80 hours. Female Moina was seen to have two eggs in a large, dark at 80 hours (Figure 2B and Figure 2C). Male *M. macrocopa* can be identified from the size of the first antenna which is longer (Fig. 2A). *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).

A B C

Picture 2. Male *M. macrocopa* (A), sexual female with sexual synthesis egg (B) and female sexual; with two eggs in hatching room (C)

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).



B

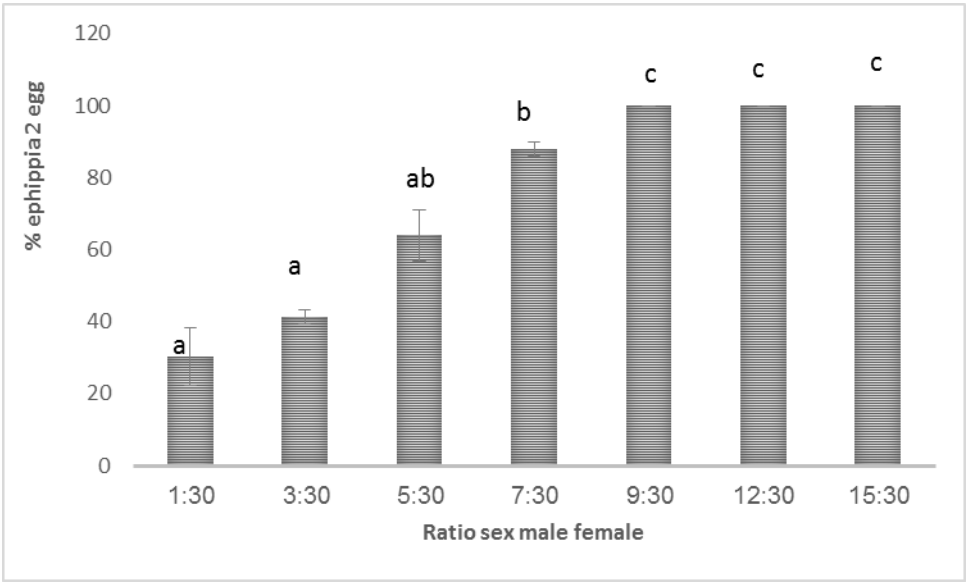


Figure 3. Percentage of ephippia to total females (A) and ephippia containing two eggs (B) in the mating of M. macrocopa using different female sex ratios from female offspring aged 70 hours. Note, different superscript on ephippia production, showed the real difference (P<0,05).

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 ºC, 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.A

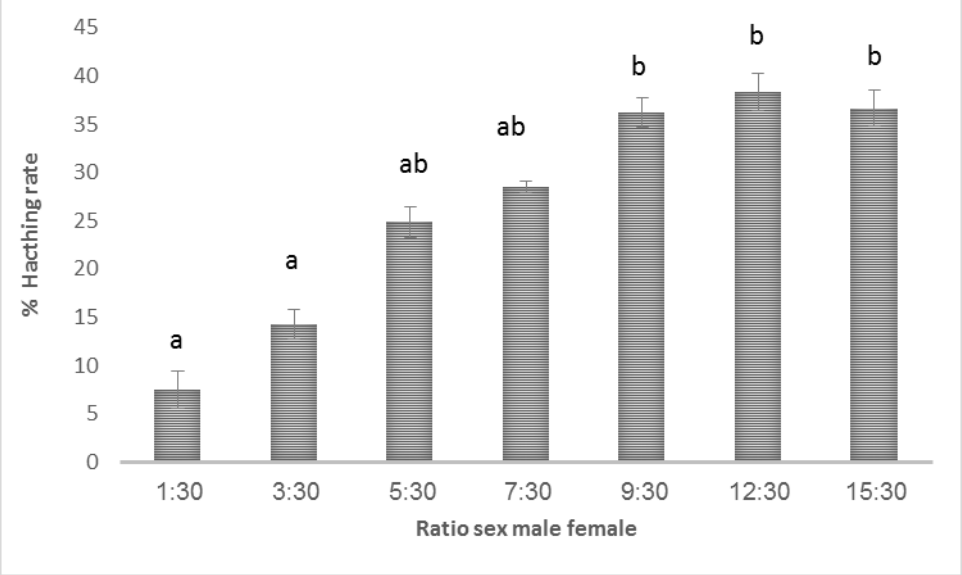


Figure 4. Percentage of hatchability of ephippia from the mating of *M. macrocopa* using different female sex ratio from female offspring aged 70 hours. Note, different superscript on ephippia,hatchability, showed the real difference (P<0,05).

Remarks, different lowercase letters on the same M. macrocopa ages, show a marked difference (P <0.05)

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

No	Parameter	Value
1	Temperature	28 – 30º 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%.

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