

QUALITY AND QUANTITY OF EPHIPIA MOINA MACROCOPA RESULTING FROM MATING USING TILAPIA FISH FECES SUSPENSION FEED

A. Sh. Mubarak

K. T. Pursetyo

A. Monica

Marine Department, Faculty of Fisheries and Marine, Airlangga University, C Campus,
Mulyorejo, Surabaya
shofy.ua@gmail.com

ABSTRACT

The needs of *Artemia* cysts in Indonesia currently reach \pm 150 tons / year and all of them are from the imports, so it can become an obstacle in process of hatching fish and shrimp in the future. Increased Culture activities have an impact on increasing fisheries waste production, including fish feces. Fish feces have been proven to be used for the Culture of *Moina macrocopa*, but have not been tested for the production for ephippia *M. macrocopa*. This study aims to examine the use of fish aquaculture/feces as feed for the quality and quantity of ephippia *M. macrocopa*. The treatment in this study was the concentration of suspension of tilapia feces 55 mg / L, 64 mg / L, and 73 mg / L and using rice bran suspension feed control 64 mg / L. The results of this study indicate that, the concentration of suspension of tilapia feces does not affect the production and hatching rate of ephippia *M. macrocopa*. *M. macrocopa* which was cultured with suspension of tilapia feces produced ephippia production and with a lower hatching rate compared to *M. macrocopa* with rice bran suspension feed.

Key words: hatching fish, cultural activity, fisheries waste.

مبارك وآخرون

مجلة العلوم الزراعية العراقية -2020: 51(عدد خاص):157-167

جودة وكمية ماكروفيوفا الناتجة عن التزاوج باستعمال علف تعليق أسماك البلطي

أحمد شوفي مبارك كوستيوان جرب بورسري أدريانا مونيكا

قسم البحرية ، كلية مصايد الأسماك والبحرية ، جامعة ايرلانغا - اندونيسيا

المستخلص

تصل احتياجات خراجات *Artemia* في إندونيسيا حالياً إلى 150 طنناً / سنوياً وجميعها من الواردات ، لذلك يمكن أن تصبح عقبة أمام عملية تفريخ الأسماك والروبيان في المستقبل. تؤثر أنشطة الاستزراع المتزايد على زيادة إنتاج نفايات مصايد الأسماك ، بما في ذلك براز الأسماك. لقد ثبت أن البراز السمكي يستخدم لثقافة *Moina macrocopa* ، لكن لم يتم اختباره من أجل إنتاج مادة *ephippia M. macrocopa*. تهدف هذه الدراسة إلى دراسة استخدام الاستزراع المائي للأسماك / البراز كعلف لجودة ونوعية مرض الأيبيبيا *macrocopa*. كان العلاج في هذه الدراسة هو تركيز تعليق براز البلطي 55 مجم / لتر ، 64 مجم / لتر ، و 73 مجم / لتر واستخدام التحكم في تغذية تعليق نخالة الأرز 64 ملغم / لتر. تشير نتائج هذه الدراسة إلى أن التركيز من تعليق براز البلطي لا يؤثر على معدل إنتاج وتفقيس من *ephippia M. macrocopa*. أنتجت *M. macrocopa* التي تم تربيتها مع تعليق براز البلطي إنتاج أبيتافيا مع انخفاض معدل الفقس .

كلمات مفتاحية : الاستزراع ، الروبيان ، نفايات ، مصايد الاسماك

INTRODUCTION

The price of *Artemia* cysts is increasing and making *M. macrocopa* as one of the choice for natural food for fish and shrimp larvae (6). *M. macrocopa* as a natural food has an advantage as they meets size requirements (± 400 um) and nutritional value comparable to *Artemia* (20), *Moina* can breed and grow rapidly, and also can be cultured using agricultural waste, livestock and food industries feed (28, 6). The utilization of *M. macrocopa* as a natural food for fish and shrimp larvae has not been maximized because the Culture of *M. macrocopa* is done like fish farming. Therefore it is necessary to find a new breakthrough so that *M. macrocopa* is no longer cultured, but using ephippia which is hatched as practically uses *Artemia* as a natural food. *M. macrocopa* Ephippia can be stored for a long time and hatched at any time (12), both as fish and shrimp larvae feed and providing inoculants in the Culture of *M. macrocopa*. *Moina* can multiply asexually (parthenogenesis) and sexually (32: 11). *Moina* sexual reproduction begins with female parthenogenesis producing male and female sexual offspring (6). *Moina*'s sexual female is a female offspring who does not reproduce parthenogenically, the eggs of a sexual female will be fertilized by males and subsequently undergo modification of carapace thickening to form ephippia (2). Our results have previously been successful in producing ephippia *M. macrocopa* with high hatchability (55%) in Culture using a combination of rice bran feed (70%) and fish meal (30%) (26). Sustainable Aquaculture development is currently a global issue. One of them is in the usage of aquaculture waste which still contains high amounts of nutrients from fish feces and excess feed for fish that has not been eaten. Aquaculture waste contains nitrogen and phosphor of 10-20% of total nitrogen in feed. In natural environments, these compounds are nutrients that are important for the growth of phytoplankton and zooplankton (16). The development of technology for the utilization of aquaculture waste can be a good asset for strengthening the potential of Indonesian fisheries in the supply of natural food, especially zooplankton, which is currently being fulfilled from imports (*Artemia* cysts).

Loh *et al.* (20,21) reported that fish feces/waste in *M. macrocopa* Culture produced a population with high density. *M. macrocopa* cultured with fish feces feed / waste gives birth at the age of 3 days and produces a high production offspring per parent (17 ind/parent). But with a lower intrinsic growth in the offspring, low intrinsic growth can inhibit reproduction of parthenogenesis (9), so that it encourages the process of gametogenesis of female offspring to produce ephippia eggs (36). The quantity and quality of ephippia *Moina* is related to the quality of nutrients received by the parent (31). Production of Ephippia and the degree of hatching of cladocera is limited by *poly unsaturated fatty acids* (PUFAs) (4) , as opposed to lower protein and amino acids that can increase the production of ephippia of *Daphnia magna* (17,18) From the analysis before it can be concluded that the problem is whether aquaculture waste/fish feces can be used for the production of good quality ephippia *M. macrocopa*. The aim of the study was to study the utilization of fish aquaculture/feces as feed for the quality and quantity of ephippia *M. macrocopa*.

MATERIALS AND METHODS

This study is using a completely randomized design (CRD) with four treatments and each treatment was repeated three times. The treatment is the concentration of tilapia feces in *M. macrocopa* mating, which is: 1) control with rice bran suspension feed 64 mg/ L, 2). Treatment A., fish feces concentration 55 mg/ L, 3). B. Treatment of feces concentration 64 mg L, 4). Treatment C. feces concentration 73 mg L.

Fish feces collection

Fish feces are obtained from tilapia (*Oreochromis niloticus*) Culture with dissolved oxygen system level of 5.00 ± 1.00 mg / L, pH 7-8 which is maintained at room temperature (29-32 ° C). Fish is fed with commercial pellet feed which is 3-5% of the weight of the fish. Fish feces is taken by chiffling (*penyiponan*) and dredging (*penyerokan*) method. Feces are collected and dried in an oven at 50 ° C for 2 days. Feces solids are stored before usage (Looh et al 2009).

Rice bran and fish feces production

Each fish's rice bran and feces are 100 g in a blender glass, then 500 mL of water added and suspended using a blender at 2000 rpm for 5 minutes twice. The second suspension is done after 30 minutes of the first suspension. Furthermore, the water suspension was filtered using a 2 mm, 0.1 mm and 40 μ m filter. The suspension that passes through the filter is added with water and the volume becomes 500 ml (25).

Analysis of protein, amino acids, fatty rice bran amino acids and fish feces

The rice bran suspension and fish feces suspension were tested using proximate based on the AOAC standard method (1995). Amino acids and fatty acids are measured using *high-performance liquid chromatography (HPLC)* from Hewlett Packard Series 1,100, based on the Fardiaz method (1989)

Provision of sexual females, males and mating of *M. macrocopa*

M. macrocopa which was used in this study came from ephippia hatching. *M. macrocopa* was adapted to rice bran suspension feed for two months in Culture with a density of 20 ind/L in a container filled with 10 L of water. Sexual female *M. macrocopa* was produced from culture with a parent density of 660 ind/L using aeration (28 ml/minute), rice bran suspension of 56-80 μ g/parent, tilapia fish *kairomone* 3.86 mg/L. *M. macrocopa* male offspring were produced from Culture without aeration with a parent density of 660 ind/L and using 45-54 μ g/mother rice bran suspension (26). Male and female offspring

were separated from the mother by screening and re-cultured with a density of 1000 ind/L with rice bran suspension feed which supporting ephippia production (36). During the preservation, concentrated rice bran suspension was given as shown in Table 1. Culture of *M. macrocopa* was carried out in a closed room with daylight illumination 700-900 lux and nighttime light 50 -100 lux. On the second day (24 hours old) the identification and separation of male offspring is carried out, especially in Culture for the supply of sexual females. Males are kept until they are 3 days old (70 hours) before being used in mating. The mating of *M. macrocopa* uses 70-hours-old female offspring, each of 30 female individuals and 9 male individuals is included in containers with a volume of one *M. macrocopa* every 2 ml water (;36) during preservation, every day 100% of the containers and water are replaced. Along with the replacement of water, the addition of feed is carried out according to each treatment

Quality and quantity of ephippia *M. macrocopa*

Collection and calculation of the amount of ephippia produced began at the age of four days *M. macrocopa* (96 hours) to six days. The quality of ephippia was identified based on the number of eggs in ephippia using a binocular microscope with 100x magnification. The percentage of ephippia in total females in mating and the percentage of ephippia containing n eggs (n is the number of eggs in ephippia) is calculated using the equation below:

$$\text{Ephippia with (n) egg (\%)} = \frac{\text{Amount of ephippia with (n)}}{\text{Total amount of ephippia}} \times 100\%$$

$$\text{Ephippia per total female (\%)} = \frac{\text{Amount of ephippia } Moina}{\text{Amount of female } Moina} \times 100\%$$

During the Culture period measurements of water quality including dissolved oxygen, pH, temperature, and total ammonia.

Hatching ability test of *M. macrocopa*

Ephippia containing two eggs was stored wet, with ephippia inserted in a micro tube (Eppendorf) containing 1 mL of distilled water. Then the micro tubes are inserted in a light-tight container and stored in a refrigerator

at a temperature of $6 \pm 1^\circ \text{C}$ (10). Ephippia from each treatment after being stored for two months, was hatched in a glass container containing 300 ml of water with an intensity of exposure light with 1800 lux (10).. *M. macrocopa* which hatched is removed and counted on the second and third days. The degree of ephippia hatching is calculated based on the equation Haghparast et al. (10). It is the

hatching index and N_i is the number of *M. macrocopa* that successfully hatched.

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

Fatty acid analysis of ephippia *M. macrocopa*

Fatty acid analysis was carried out on ephippia produced from Culture with rice bran suspension feed (control), fish feces (A), and feces with fish oil emulsion (B). Ephippia produced from Culture with an initial density of 20 ind/L with a volume of 10 ml of water. On sixth days *Moina* is fed with rice bran suspension. And on the sixth to twelfth day were given feed according to the treatment. On the eighth day ephippia was harvested. Ephippia is harvested by turning/flipping water so that ephippia and dirt accumulate in the middle of the tub. Ephippia is separated from feces and washed three times before storage (24). Analysis of fatty acid ephippia *M. macrocopa* carried out by using 10 grams of each (wet weight) based on the Fardiaz (1989) method using Shimadzu GC-2010 Gas Chromatography

Data Analysis

Data from the observations were analyzed using ANOVA. If the results of variance analysis are known that the treatment shows results that are significantly different or very really different, then Duncan's Multiple Range Test is continued to find out the treatment with the best response at 95% confidence level.

RESULTS AND DISCUSSION

Male *M. macrocopa* offspring can be easily identified after 24 hours of age based on their smaller size and the presence of the first antenna which is longer than the female *M. macrocopa* (Figure 1A). Sexual females have the same morphology as parthenogenetic females, sexual females can be identified after synthesizing eggs in ovaries with a large size and darker color at the age of 70 hours (Figure 1B). Mating of tilapia using the ratio of 9: 30 female male sex.

Survival rate of *M. macrocopa*

The suspension of tilapia feces was carried out during the mating of *M. macrocopa* with a concentration of 55 mg / L dissolved organic matter, 64 mg / L, and 73 mg / L. The survival rate of *M. macrocopa* during mating is not

affected by feed treatment after first and second ephippia production (Figure 2.). The survival rate of *Moina* after the third highest day of Culture on mating was a rice bran suspension of $80.8 \pm 3.2\%$.

Ephippia production

Usage of tilapia feces suspension caused a decrease in ephippia production and ephippia percentage of total females in mating (figure 3A). The concentration of tilapia feces suspension used did not cause a difference in the percentage of ephippia in females in mating. The percentage of ephippia in total female *M. macrocopa* in mating using tilapia feces suspension was 69-75%, while those using rice bran suspension feed were $88.84 \pm 2.8\%$. The substitution of rice bran suspension feed with tilapia feces suspension did not cause a significant difference in the percentage of ephippia containing two eggs. Reduction of the suspension feed concentration of tilapia feces (55 mg/L) caused a decrease in ephippia containing two eggs to $89.58 \pm 4.7\%$. The mating of *M. macrocopa* with a 9:30 female male sexual ratio produces ephippia containing two eggs which is 89.58- 100% high (Figure 3B).

Hatching rates

M. macrocopa mating using feed suspension of tilapia feces produces ephippia with lower hatching rates compared to mating using rice bran suspension feed. Ephippia mating results with rice bran suspension feed have a hatching rate of $32.50 \pm 1.18\%$ while ephippia of marital results with a suspension of tilapia feces with the same concentration of $20.33 \pm 2.36\%$. The increase and decrease in feces concentration of tilapia did not affect the degree of ephippia *M. macrocopa* hatching (Picture 4.). Tilapia feces contain relatively high protein of 16.58% but with a low fat content of 1.84%. Rice bran suspension contains fat (10.54%) which is higher than the suspension of tilapia feces (1.84%) (Table 2). Rice bran having high rice bran content was high in n-6 (linoleic, arachidonic, hecocentric) fatty acids (28.48%), but with low omega 3 fatty acids (0.97). Tilapia feces detected contain low omega-3 but contain EPA (0.14%) and DHA (0.2%) and total omega-6 concentrations of 12.22%. The concentration of protein, amino acids and fat in feed affects the fecundity or

production of offspring per parent and the speed of embryo development in cladocera (18). The fat concentration in feed (*D.magna*) affects the allocation of energy from metabolism for reproduction and growth. Cholesterol is fat which is the precursor to the formation of micro-crustacean hormones (27), increasing somatic growth, while PUFAs play a role in reproduction (34), cholesterol needs must be met from feed because cladocera cannot be synthesized alone. Loh *et al.* (20, 21) reported that fish feces / waste in *M. macrocopa* Culture produced a population with high density with long duration of culture because of the lower intrinsic growth of moina offspring. Low intrinsic growth can inhibit reproduction of parthenogenesis (9) and encourage the process of gametogenesis of female offspring to produce ephippia eggs (36). Our results show that Moina's mating using suspension food of tilapia feces produces low ephippia. This low ephippia production is thought to be due to the low fat content in fish feces (1.84%), because the quality and quantity of ephippia production in cladocera is influenced by the concentration of fat and fatty acids in feed (31). Production of ephippia cladocera is limited by the availability of *poly unsaturated fatty acid* (PUFAS) (4), including DHA and linolenic acid concentrations in feed (14), tilapia feces contain omega-3, EPA and DHA (0.94%) which is lower and omega-6 is 12.22%, which is lower than the content of n-6 fatty acids (linoleic, arachidonic, hecosentrionate) rice bran suspension of 28.48% in the control. Our results also show that fatty acid concentrations in feed which correlate with fatty acids in ephippia *M. macrocopa*. Married ephippia *M. macrocopa* from fish suspension feed has omega-3 of 0.55% and omega-6 of 20.48%, which is lower than the concentration of omega-6 in cultured ephippia with rice bran suspension feed (Table 2). Mated Ephippia *M. macrocopa* using rice bran suspension feed has a percentage of omega-6 acid of 26.90% and omega-3 acid concentration of 0.50%. Mated Ephippia *M. macrocopa* using feces of tilapia feces suspension had omega-6 acid percentage of 20.48% and 0.54% omega-3 acid concentration (Table 3.). Omega-3 fatty acids play an important role in the development and

function of the central nervous system and neurogenesis in the brain, as well as cell division and embryo development (3; 35), where omega-3 fatty acid diets, omega-6 affects neurogenesis in the brain (*Homarus americanus*) and growth speed related to the number of cells entering the S phase (19). In addition, the presence of omega 3 and omega 6 has been shown to increase egg hatchability and survival as in *Artemia*, fish and crustaceans (30, 15). Low percentage of omega-6 in ephippia *M. macrocopa* with fish feces is suspected to be the cause of the decrease in ephippia hatching rate even though Ephippia with suspension food of tilapia feces contains EPA fatty acids (0.55%). Linoleic acid and α -linolenic acid, and both of these fatty acids are essential fatty acids for cladocera (22; 29). Some cladocera species are reported to be able to synthesize EPA and DHA from α -linolenic acid, but with different synthesis capabilities (23). Tilapia feces has the potential as a feed for the production of ephippia *M. macrocopa* with increased availability of PUFAS through the addition of fish oil emulsions that are rich in EPA and DHA. Supplementation of PUFA in algae has been shown to increase ephippia production (1) and ephippia *D magna* hatching rate (33). During the male mating Moina only pursues and populates sexual females in a synthesizing eggs condition (over 70 hours old). The duration of mating lasts for 8-10 minutes (8). Mating of *M. rice branchiata* consists of three phases which begin with the male capturing the sexual female, then followed by the male movement to position themselves until perpendicular to the sexual female, then copulation which lasts for 16-25 seconds(7). The male and female sex ratio of *M. macrocopa* affected more the number of ephippia containing two eggs compared to the concentration of suspension of fish feces. Conde *et al.* (5) stated that, Moina's sexual female if not fertilized by a male will produce ephippia without eggs or damaged. This mating using the sexual ratio of male and female sexual *M. macrocopa* which is 9:30, that in order will producing ephippia containing two eggs at 100%, and lower than the sex ratio of female male *M. australiensis* at 4: 5 (13). Male *M. macrocopa* can marry more sexual females.

Water quality during mating of *M. macrocopa* includes water temperatures 29-32° C, water pH 7.5-8, hardness of 60 mg / L CaCO₃.

Dissolved oxygen at the beginning of the study was 4 mg / L and decreased at the end of the study to 2.8 mg / L (Table 4.).

Table 1. Concentration of rice bran suspension for the induction of production of male, female sexual and maintenance of *M. Macrocopa* offspring. (26).

Day	Rice bran Suspension (mg/L)		
	Male Induction	Ehipia Induction	Offspring Preservation
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

Table 2. The concentration of protein, fat, amino acids (% w / w) of tilapia rice bran and feces

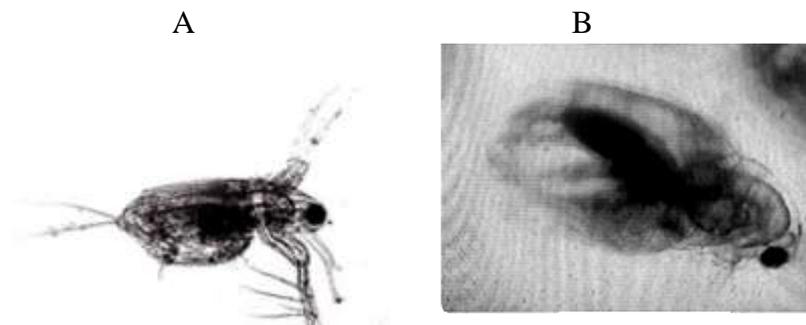
Parameter	Rice bran	Fish feses
	(% w/w)	
Protein	12,16	16,27
Fat	10,52	1,84
Carbohidrate	68,82	18,27
Essential amino acid (%)		
Leusin	4.29	6,377
Arginin	3.82	4,288
Lisin	2.11	3,408
Histidin	1.61	1,429
Valina	3.03	4,508
Fenilalanin	2.89	3,628
Treonin	2.33	3,299
Metionin	1.27	1,154
IsoJeusin	2.13	3,793
Non-esensial amino acid		
Glutamat	8.61	9,731
Aspartat	4.80	6,927
Glisin	3.17	5,223
Serin	2.69	3,738
Alanin	4.31	5,278
Tirosin	3.42	2,199

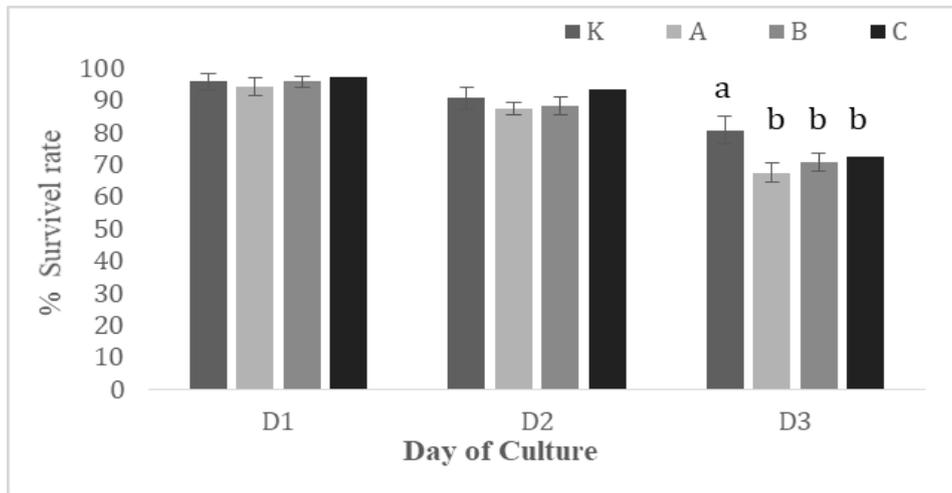
Table 3. Concentration of fatty acid ephippia *M. macrocopa* (% w / w fat) from Culture using tilapia feed suspension and rice bran suspension

NO	Fatty acid	<i>Ephippia M. macrocopa</i>		Rice bran	Fish Feces
		rice bran	Fish feces		
1	12:0	0,17±0,01	0,16±0,02		0,14
2	13:0	0,04±0,01	0,04±0,01		
3	14:0	1,25±0,02	1,41±0,04	0,3	0,88
4	15:0	0,30±0,05	0,31±0,02	0,02	0,16
5	16:0	18,78±0,38	16,9±0,41	14,60	15,53
6	16:1n-7	5,89±0,11	4,52±0,35	0,01	
7	17:0	0,12±0,01	0,15±0,01	0,03	0,22
8	18:0	2,37±0,20	2,60±0,31	1,21	3,00
9	18:1n-7	0,05±0,01	0,04±0,01		
10	18: 1n-9	28,37±1,10	21,22±2,34	31,83	22,98
11	18:2n-6	23,65±1,04	17,30±1,30	28,48	12,19
12	20:0	0,11±0,01	0,13±0,04	0,04	0,26
13	18:3n-6	0,2±0,01	0,15±0,01		0,03
14	20:1	0,25±0,06	0,20±0,02		0,41
15	18:3n-3	0,5±0,08	0,5±0,04	0,97	0,61
16	20:2	0,06±0,01	0,07±0,02	0,04	0,19
17	22-0	0,22±0,01	0,21±0,02	0,26	0,27
18	20:3n-6	0,05±0,02	0,02±0,01		
19	20:5n-3		0,04±0,01		0,13
20	22:6n-3				0,20
21	Total n-3	0,5	0,54	0,97	0,94
22	Total n-6	26,90	20,48	28,48	12,22

Table 4. Water quality of mating culture media for *M. macrocopa* using rice bran suspension feed and substitutes for suspension of fish meal with different percentages

No	Parameter	Value
1	Temperature (° C)	29-31
2	pH	7,5-8
3	Hardness (mg/L CaCO ₃)	60
4	Dissolved oxygen (mg/L)	4,0 -2,7
5	Ammoniac total (mg/L)	<0,250

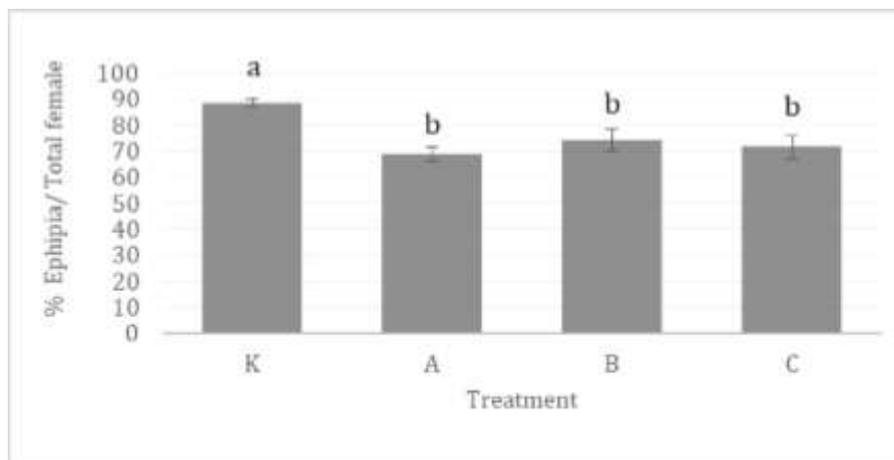
**Picture 1. Morphology of male *M. macrocopa* (A) dan female sexual with egg synthesizing (B).**



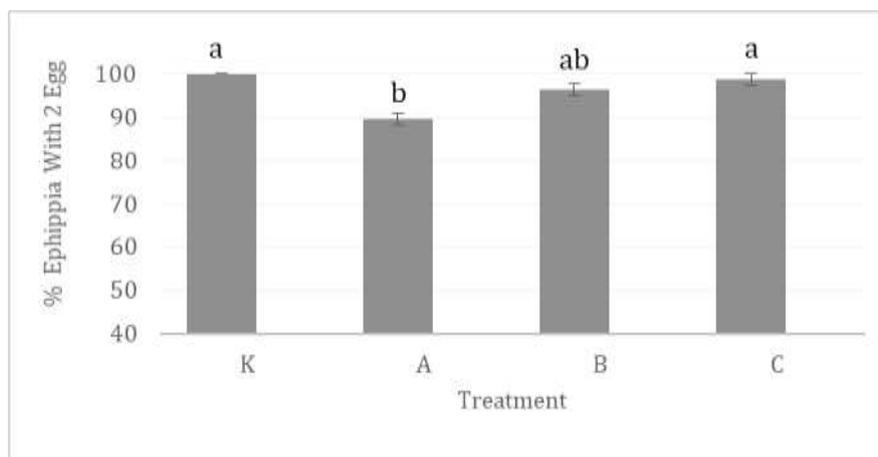
Picture 2. The survival rate of *M. macrocopa* in mating uses 70-hour-old offspring using suspension feed of tilapia feces

Notes: K; rice bran suspension feed 64 mg/L A; feces suspension feed 55 mg/L, B feces suspension feed 64 mg/L, dan C feces suspension feed 73 mg/L

A

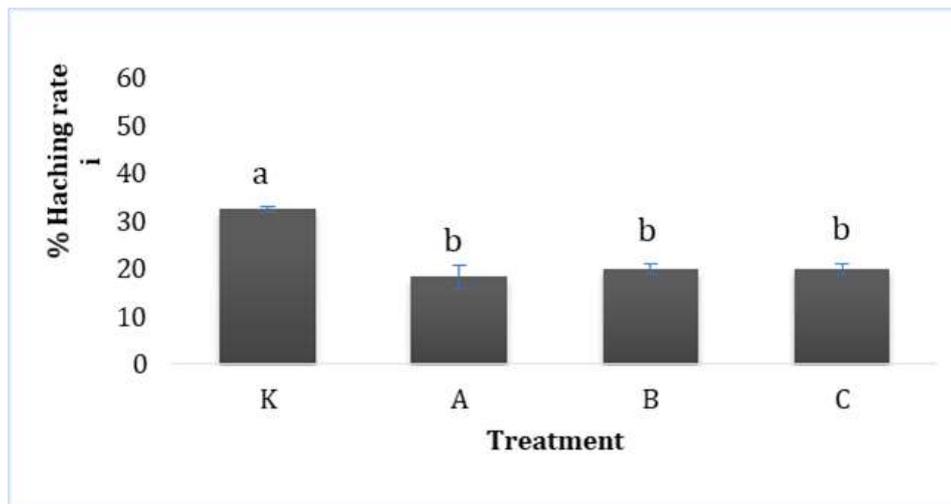


B



Picture 3. Percentage of ephippia production per female total (A) and the percentage of ephippia containing two eggs (B) marital results of *M. macrocopa*

Notes K; rice bran suspension feed 64 mg/L A; feces suspension feed 55 mg/L, B feces suspension feed 64 mg/L, dan C feces suspension feed 73 mg/L



Picture 4. The degree of marital for ephippia *M. macrocopa* from hatching using suspension feed of tilapia feces

Notes K; rice bran suspension feed 64 mg/L A; feces suspension feed 55 mg/L, B feces suspension feed 64 mg/L, dan C feces suspension feed 73 mg/L

Suspension concentration of tilapia feces did not affect the production and hatching rate of ephippia *M. macrocopa*. *M. macrocopa* cultured using suspension of tilapia feces producing ephippia with a lower hatching rate compared to *M. macrocopa* with rice bran suspension feed.

ACKNOWLEDGEMENTS.

The authors are grateful to Government of The Republic of Indonesia for all support to this research. Also we thank the editor and reviewer for their suggestion, which improved our manuscript

REFERENCES

1. Abrusan G, P. Fink, and W. Lampert 2007. Biochemical limitation of resting egg production in daphnia. *Journal Limnology Oceanography* 52: 1724-1728
2. Alekseev V, B. Stasio, and J. Gilbert 2007. Diapause in aquatic invertebrates: Theory and human use. Springer Science & Business Media. 1- 214
3. Beltz B.S, M. F. Tlusty, J. L. Benton, and D.C. Sandeman. 2007. Omega-3 fatty acids upregulate adult neurogenesis. *Neuroscience Letters* 415:154-158
4. Choi J, S. Kim, L.G. Hwan, 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 and Evolution* 6 : 2.817- 2.832
5. Conde P.J, F. Valdés, S. Romo, and C. Pérez 2011. Ephippial and subitaneous egg abortion: relevance for an obligate

parthenogenetic daphnia population. *Journal Limnology* 70: 69-75

6. Dodson S, C. Caceras, and C. Rogers 2010. Ecology and classification of North American freshwater invertebrates. Chapter 20. Cladocera and other rice branchiopoda Third Edition. San Diego California. Academic Press: 775-827

7. Forro L. 1997. Mating behaviour in *Moina brachiata* (Crustacea: Anomopoda). *Arch. Hydrobiology*. 360

8. Freedberg S, and D. R. Taylor, 2007. Sex ratio variance and the maintenance of environmental sex determination. *Journal Evolutionary Biology* 20: 213- 220

9. Frost P. C, D. Ebert, J.H. Larson, M.A. Marcus, M.D. Wagner, and A. Zalewski 2010. Transgenerational effects of poor elemental food quality on *Daphnia magna*. *Oecologia* 162: 865-887

10. Haghparast S, A. Shabani, B. Shabanpour, and S.A. Hoseini 2012. Hatching requirements of *Daphnia magna* Straus, 1820, and *Daphnia pulex* Linnaeus, 1758, diapausing eggs from Iranian populations *in vitro*. *Journal Agriculture Sciences Technology* 14: 811-820

11. Hiruta C, C Nishida, and S.Tochinai 2010. Abortive meiosis in the oogenesis of parthenogenetic *Daphnia pulex*. *Chromosome Research* 18: 833-840

12. Hiruta C, and S. Tochinai 2014. Formation and structure of the ephippium (resting egg case) in relation to molting and egg laying in the water flea *Daphnia pulex* De Geer

- (Cladocera: *Daphniidae*). Journal of Morphology 275: 769 -767
13. 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
14. Jonasdottir S. H, A.W. Visser¹, and C. Jespersen 2009. Assessing the role of food quality in the production and hatching of *Temora longicornis* eggs. Marine Ecology Progress 382: 139-150
15. Kangpanich C, J. Pratoomyot, N. Siranonthana, and W. Senanan 2016. Effects of arachidonic acid supplementation in maturation diet on female reproductive performance and larval quality of giant river prawn (*Macrobrachium rosenbergii*). Peer Journal, Biological and Medical Science. DOI 10.7717/peerj.2735
16. Kibria G., D. Nugegoda., R. Fairclough, and P. Lam 1997. The nutrient content and the release of nutrients from fish food and faeces. *Hydrobiologia* 357: 165-171
17. Koch U, D. Creuzburg, P. Grossart, and D. Straile 2011, Single dietary amino acids control resting egg production and affect population growth of a key freshwater herbivore, *Oecologia* 167: 981-989
18. Koch U, E. Elert, and D. Staile 2009. Food quality triggers the reproductive mode in the cyclical parthenogenesis daphnia (Cladocera). *Journal Oecologia* 159: 317-324
19. Koopman H, and Z. A.Siders 2013. Variation in egg quality in blue crabs, *Callinectes sapidus* from North Carolina: does female size matter? *Journal of Crustacean Biology* 33: 481-487
20. Loh J.H, H.K. Alan, Y.S. Hii, T. J. Smith, and L.G. Khoo, 2013. Impact of Potential Food Sources on the Life Table of the Cladoceran, *Moina macrocopa*. The Israeli Journal of Aquaculture – Bamidgeh 65, 1-8
21. Loh J.H, C.W. How, Y.S. Hii, G. Khoo, and H.K.A. Ong,. 2009. Fish faeces as a potential food source for cultivating the water flea, *Moina macrocopa*. *Journal of Science and Technology in the Tropics* 5: 5-10
22. Lomthaisong K, and L. Sanoamuang 2012. Biochemical composition of three species of fairy shrimp (Branchiopoda: Anostraca) from Thailand. *Journal of Crustacean Biology* 32:81-87
23. Masclaux H, A. Bec, M. K. Kainz, F. Perrie, C. Desvillettes, and G. Bourdier 2012. Accumulation of polyunsaturated fatty acids by cladocerans: effects of taxonomy temperature and food. *Freshwater Biology* 57: 696-703
24. Mubarak A.S. (1), D. Jusadi , M. Jr Zairin, and M.A. Suprayudi 2017 . Evaluation of the rice rice bran and cassava suspension use in the production of male *Moina macrocopa* offspring and ephippia. *AAFL Bioflux*, Volume 10, Issue 3
25. Mubarak A.S. (2), D. Jusadi , M. Jr Zairin, and M. A. Suprayudi 2017. The population growth and the nutritional status of *Moina macrocopa* feed with rice rice bran and cassava rice bran suspensions. *Jurnal Akuakultur Indonesia* 16 (2), 223–233
26. Mubarak AS(3), D. Jusadi, M. Jr Zairin, and M. A. Suprayudi 2017. Production of *Moina macrocopa* Ephippia through Feed Manipulation, Density, Fish “Kairomone”, and Dissolved Oxygen. Disertation Doktorat at Institut Pertanian Bogor (IPB) Bogor. Indonesia
27. Nagaraju G. 2011. Review reproductive regulators in decapod crustaceans: an overview. *The Journal of Experimental Biology* 214: 3-16
28. Patil S.S, A. J. Ward, M. S. Kumar, and A.S. Ball 2010. Utilizing bacterial communities associated with digested piggery effluent as a primary food source for the batch culture of *Moina australiensis*. *Bioresource Technology* 101:3371-3378
29. Persson J, and T. Vrede 2006. Polyunsaturated fatty acids in zooplankton: variation due to taxonomy and trophic position. *Freshwater Biology* 51: 887-900
30. Prusinska M, O. Kushniryk, O. Khudyi, L. Khuda, and R. Kolman 2015. Impact of enriching larval brine shrimp (*Artemia* sp.) with a supplement containing polyunsaturated fatty acids on their growth and mortality. *Archives of Polish Fisheries* 23: 149-154. DOI 10.1515/aopf-2015-0017
31. Putman A, C. D. Martin, B. Panis, and L. De Meester 2015. A comparative analysis of the fatty acid composition of sexual and asexual eggs of *Daphnia magna* and its

plasticity as a function of food quality. *Journal Plankton Research* 37: 752-763.

32. Simon J, F. Delmotte , C . Risp, and T. Crease 2003. Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. *Biological Journal of The Linnean Society* 79: 151-163

33. Sperfeld E, and A.Wacker 2011. Temperature affects limitation of *Daphnia magna* by *eicosapentaenoic acid* and the fatty composition of body tissue and eggs. *Freshwater Biology* 12: 1365-8427

34. Wacker A, and M. D. Creuzburg 2007. Allocation of essential lipids in *Daphnia*

magna during exposure to poor food quality. *Functional Ecology* 21:738-747

35. Watters C, S. Iwamura, H. Ako, and F.H. Deng, 2012. Nutrition Considerations in Aquaculture: The Importance of Omega-3 Fatty Acids in Fish Development and Human Health. *Foods and Nutrition FN-11*

36. Zadereev E, and E. Lopatina 2007. The induction of diapause in *Moina* by species-specific chemical cues. *Aquatic Ecology* 41: 255–261