Maximizing production of a male offspring in Moina macrocopa culture through manipulation of rice bran suspension concentration

A S Mubarak1,3, D Jusadi2, M Zairin Jr.2 and M A Suprayudi2

1Aquaculture Study Program of the Faculty of Fisheries, Airlangga University, Campus C Mulyorejo Surabaya, East Java 60113
2Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural Institute, Campus of IPB Dramaga Bogor, West Java 16680
3Corresponding author: shofy.ua@gmail.com

Abstract. The quality and quantity of Moina ephippia is influenced by the male and female sexual ratios in the population. The availability of males in the population can increase the number of fertilized eggs and ephippia hatching rates. This study aims to examine the concentration of rice bran suspension in culture to produce the most male offspring, and the quality and quantity of ephippia produced. Moina was cultured for twelve days and from the fifth day the feed is given with different concentrations. The results of this study indicate that, Moina culture using bran suspension feed with a concentration of 0.36-0.48 ml/L starting on the fifth day resulted in the production of low calf offspring but produced the highest total production of male offspring of 818 ± 35 ind/L. Increased production of male offspring increases the production of ephippia containing two eggs but does not increase the degree of hatching significantly.

1. Introduction
The use of ephippia Moina as a natural food source has an advantage that it can be hatched at any time and can be stored for a long time [1]. The quality and efficacy of ephippia Moina are influenced by male and female sexual ratios in the population. High concentration of bran suspension in the feed would reduce the production of male offspring in the population which reduces ephippia quality and the degree of ephippia hatching [2, 3]. Efforts to increase the production of Moina male offspring can be done by decreasing feed concentrations in aquaculture with high population density and supporting the production of male offspring [4, 5] Reduction and addition of rice bran suspension concentrations in Moina culture with high population density (fifth day) will affect the production of male offspring.

Rice bran suspension contains 11.16% protein, 10.62% fat, Moina culture uses bran suspension food 3 ml/L - 4.5 ml/L on the first day until the fifth day, resulting in the production of male offspring of 6.10% of the total offspring born [2]. The rice bran suspension feed concentration has implications for the availability of protein, fat and parent amino acids, which affect the production of offspring (fecundity) and the rate of development of eggs into offspring, and also had an implications for changes in reproductive patterns from asexual to sexual which begins with the formation of male offspring [6, 7].

The male production of M. macrocopa americana increases when high density of population is followed by a sudden decline in the amount of feed available [8]. Reducing the concentration of rice
bran suspension at certain concentrations will increase the production of male offspring, but the reduction in the concentration of higher bran suspensions will reduce *Moina*’s energy status so that it is insensitive to stress pressure due to high population [9]. The production of male caldosera is also influenced by the parent experience [10], which including the concentration of rice bran suspension at the beginning of the culture period. This study aims to examine the concentration of rice bran suspension needed to produce the most *Moina* male offspring in culture and to determine the quality and quantity of ephippia produced.

2. Materials and methods

2.1. Research design

This study uses a completely randomized design (CRD). *Moina* is cultivated using bran suspension (0.3 mL/L first day to 0.4 mL/L on the fourth day [2] and from the fifth day the feed is given a different concentration, ie treatment A with bran suspension concentration of 0, 45-0.6 mL/L, A1 treatment of bran suppression with concentration as much as 0.41-0.54 mL/L, A2 treatment of bran concentrated with concentration of 0.36-0.48 mL/L, treatment A3 concentration of bran suspension as much as 0.32-0.42 mL/L and A4 treatment, the concentration of bran suspension was 0.50-0.66 mL/L. Each treatment was repeated four times. The feed concentrations for the eighth to twelfth days for each treatment were the same (Table 1).

| Table 1. Feed concentration of bran suspension on the culture of *M. macrocopa* male induction treatment group (mL/L). |
|---|---|---|---|---|
| Culture days | A | A1 | A2 | A3 | A4 |
| First | | | | | |
| Second | | | | | |
| Third | | | | | |
| Fourth | | | | | |
| Fifth | 0.45 | 0.41 | 0.36 | 0.32 | 0.50 |
| Sixth | 0.49 | 0.44 | 0.39 | 0.34 | 0.54 |
| Seventh | 0.54 | 0.49 | 0.43 | 0.38 | 0.59 |
| Eighth until Twelfth | 0.60 | 0.54 | 0.48 | 0.42 | 0.66 |

2.2. Culture media

The *Moina* culture media in this study is using water from the processed river water collected from water reservoirs, which belong to the Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural Institute. Water from the reservoir is put into a 1000 L fiber tub and aerated at least three days before being used for *Moina* culture. Water from the fiber tub was filtered with a 40 µm sieve before being inserted into a trial container to eliminate competing zooplankton.

2.3. The making of bran suspension

The bran suspension is made by dissolving as much as 100 g of bran in 500 mL of water, then suspended with a blender for five minutes (2000 rpm). After leaving it for 30 minutes then suspended again using a blender for five minutes, then filtered three times using a 2 mm, 0.1 mm and nylon 40 µm filter. The suspension which obtained than added with water so that the volume becomes 500 mL. This bran suspension has a concentration of 72 mg / L dissolved organic matter, 0.83% protein, and 0.79% fat [11]

2.4. Provision of inoculants and culture of *M. macrocopa*

*Moina* used in this study was obtained from waters in the Surabaya region, then cultivated individually (one *Moina*20 mL) in several generations to obtain species that have the best growth and production performance of offspring. Furthermore, *Moina* is cultured with bran suspension feed for 2 months at a
density of 20 / L volume of 10 L water. The cultivated Moina offspring are now becomes inoculants in this study with the same initial density [12].

Moina culture in this study was conducted for 12 days. In the first four days of culture, the amount of feed given for all treatments was the same. Starting on the fifth day, the concentration of bran suspension for each treatment is different as shown in Table 1. Feed is given twice a day at 08.00-09.00 WIB and 19.00 - 20.00 WIB (Standart Indonesian Western Time), each as much as 50% of daily concentration.

During the culture period, the container is replaced every two days, while water changes are carried out from the fifth day to the eleventh day to prevent the formation of phylum layers (formed from the rest of feed and feces) that can ensnare and causing Moina's death. Water in containers is disposed of as much as 33% of the volume of culture media, then water and moina are transferred to new containers, and as much water is removed so that the volume of water remains 10 L. During the culture period each container is aerated at 319 ml/minute [11].

2.5. Observation results

2.5.1. Male offspring production

Aeration stopped than 15 minutes later sampling was done for moina by taking 100 ml of water from five different points. Sampling is done to calculate the total amount of Moina. Sampling is done from the second day to the twelfth day. Along with the sampling, adult moina were taken which were ready to give birth to offspring (20-40 ind), then kept in different containers with a density of 66 ind / L to give birth to offspring. Moina's offspring are separated from the mother and cultivated for 24 hours, then counting the number of offspring and identifying the sex of the offspring using a binocular microscope (with 100x magnification). These results are used for the calculation of offspring production per parent and the percentage of male offspring using the equation below

\[
\text{Offspring Prod/Parent} = \frac{\text{Total Male } Moina \text{ Children} - \text{Total Female } Moina \text{ Parent}}{\text{Total Male } Moina \text{ Children} \times 100%}
\]

\[
\text{Male Percentage} = \frac{\text{Total Male } Moina \text{ Children}}{\text{Total } Moina \text{ Children}} \times 100%
\]

The ephippia collection is carried out from the fifth day to the twelfth day along with the replacement of the container. The ephippia collection begins by turning the water in the tub so that all the remaining feed and ephippia gather in the center of the container. The rest of the feed and ephippia are taken with a pipette, then put in the petri dish. The next process is to rinse with water so that ephippia can be seen. The Ephippia is removed in a new petri dish then washed three times in the same way before being identified. Furthermore ephippia are identified by a binocular microscope (with 100x magnification). The percentage of ephippia containing two, one and without eggs is calculated based on the following equation

\[
\% \text{ ephippia with } (X) \text{ egg} = \frac{\text{Amount of ephippia with } (X) \text{ egg}}{\text{Amount of total ephippia}} \times 100%
\]

Notes: X is amount of eggs in ephippia.

2.5.2. Ephippia Moina hatchability test
Ephippia *Moina* (containing two eggs) is stored in wet condition, by entering ephippia in eppendorf which contains water (aquadess) with a density of 200 grains/ml. Then Eppendorf is put in a light-tight container and stored at a temperature of 5 ± 1° C in a refrigeration cabinet [13]. Fifty grains of ephippia Moina (containing two eggs) from each treatment were stored for two months, then hatched in a glass container containing 300 ml of water with an intensity of exposure of 1800 lux [13]. Moina Ephippia hatches after 36 hours. Moina which has hatched is then transferred and counted in the number. The value of hatchability ephippia Moina is calculated based on equation [13]. Where the I is the hatching index and Ni is the number of larvae that hatch.

\[
Hatching \ rate = \frac{15}{3} \sum \frac{N_i}{N_e} \times I_i
\]

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

2.6. Data analysis

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

3. Results and discussion

3.1 Results

Culture of *Moina* using bran suspension feed with a concentration of 0.45-0.6 mL/L (A) and 0.5-0.66 mL/L (A4) on the fifth day to twelfth producing the highest population peaks of 14,930 ind/L and 15,373 ind/L (Figure 1 and Table 2). The decrease in bran suspension concentration caused a decrease in the peak population of Moina, with the lowest peak population in bran suspension -feeding as much as 0.32-0.42 mL/L (A3) as many as 6,973 ind/L (Figure 1.).

![Figure 1](image_url)

**Figure 1.** Population of *M. macrocopa* in culture with different bran suspension concentrations starting on the fifth day, as much as 0.45 - 0.6 mL/L (A), 0.41-0.54 mL/L (A1), 0.36-0.48 mL/L (A2), 0.32-0.42 mL/L (A3) and 0.50-0.66 mL/L (A4). Different lowercase letters on the same sampling day showed significant differences (P<0.05).
Figure 2. Production of offspring per mother *M. macrocopia* in culture with different bran suspension concentrations starting on the fifth day, as much as 0.45-0.6 mL/L (A), 0.41-0.54 mL/L (A1), 0.36-0.48 mL/L (A2), 0.32-0.42 mL/L (A3) and 0.50-0.66 mL/L (A4). Different lowercase letters on the same sampling day showed significant differences (P<0.05).

The population and production of offspring per mother *Moina* on the fourth day (8.32-8.59 ind) did not differ significantly between treatments. Reduction of the fifth day bran suspension concentration from 0.45 mL/L to 0.36 mL/L (A2) caused a significant decrease in offspring production, ie the lowest from other treatments 2.22 ind/parent (Figure 2.) and produced the production of male offspring the highest is 13.48% of the total production of *Moina* offspring (Figure 3.) in the population of 5,840 ind/L. Reduction of the fifth-day bran suspension from 0.45 mL/L to 0.32 mL/L (A3) caused a decrease in the production of male offspring to be as much as 4.93% of the total production of *Moina* offspring (Figure 3.).

Figure 3. Production of *M. macrocopia* male offspring in culture with different bran suspension concentrations starting on the fifth day, 0.45-0.6 mL/L (A), 0.41-0.54 mL/L (A1), 0.36-0.48 mL/L (A2), 0.32-0.42 mL/L (A3) and 0.50-0.66 mL/L (A4). Different lowercase letters on the same sampling day showed significant differences (P<0.05).
Reduction of the sixth-day bran suspension concentration from 0.49 mL/L to 0.39 mL/L (A2) resulted in the production of *Moina* male offspring as much as 10.6% of the total offspring, but not as different from treatments A1, A3 and A4. Reduction of the seventh-day bran suspension concentration from 0.54 mL/L to 0.43 mL/L (A2) resulted in the production of *Moina* males as much as 6.45% of total offspring production, but not different from treatments A1, A3 and A4.

Culture of *Moina* using bran suspension feed with a concentration of 0.36-0.48 mL/L (A2) on the fifth day to twelfth produced the highest total production of *Moina* male offspring as much as 818±35 ind/L. The culture of *Moina* with lower bran suspension feed from 0.32 to 0.42 mL/L (A3) produced the lowest total production of male offspring of 292±48 ind/L.

*Moina* begins to produce ephippia starting on the seventh day with the highest daily production on a ninth day (Figure 4.). Culture of *Moina* using bran suspension feed with a concentration of 0.5-0.66 mL/L (A4) on the fifth day to twelfth produced the highest ephippia production of 410±20 grains/L. Reduction of bran suspension by 30% from concentration A, namely the concentration of bran suspension 0.32-0.42 mL/L (A3) on the fifth day to twelfth, resulted in the lowest production of ephippia *Moina* as much as 122±3 grains/L (Table 2).

**Figure 4.** Production of ephippia *M. macrocopa* in culture with different bran suspension concentrations starting on the fifth day, as much as 0.45-0.6 mL/L (A), 0.41-0.54 mL/L (A1), 0.36-0.48 mL/L (A2), 0.32-0.42 mL/L (A3), and 0.50-0.66 mL/L (A4). Different lowercase letters show significant differences (P<0.05).

**Table 2.** Production of male offspring, quantity, and quality of ephippia *M. macrocopa* in culture with different bran suspension concentrations starting on the fifth day.

| Parameter                      | Male induction |
|-------------------------------|----------------|
|                               | A              | A1             | A2            | A3            | A4            |
| Population on seventh (L)     | 14960±269d     | 12937±804c     | 11117±796b    | 6973±184a     | 15373±345d    |
| Ephippia production (grain/L) | 288±29c        | 265±31c        | 173±27b       | 122±3a        | 411±20d       |
| % ephippia/total population   | 1.93           | 2.05           | 1.55          | 1.74          | 2.67          |
| Male on population (L)        | 546±63b        | 542±85b        | 818±35c       | 292±48a       | 572±117b      |
| Ephippia with 2 (%)           | 83.00±4.36b    | 84.67±1.15bc   | 89.00±1.73d   | 77.00±1.00a   | 87.33±1.15cd  |
| Ephippia with 1 (%)           | 11.33±3.51a    | 11.67±1.53a    | 10.00±2.00a   | 17.00±3.61b   | 11.00±1.00e   |
| Empty ephippia (%)            | 5.67±2.89ab    | 3.67±0.58a     | 1.00±1.00ab   | 6.00±4.36b    | 1.67±0.58ab   |
Hatchability (%)  

| Potential born Moina (L) | 14.67±2.02a | 10.67±0.76a | 16.33±2.51a | 11.50±3.04a | 10.83±2.47a |

Notes: Lowercase that are different in one parameter show significant differences (P<0.05).

The resulting Ephippia consists of three categories, namely ephippia containing 1, 2, and without eggs. The highest Ephippia contains two eggs produced from Moina culture using bran suspension feed with concentrations of 0.36-0.48 mL/L (A2) and 0.5-0.66 mL/L (A4) as much as 89% and 87%. The concentration of bran suspension did not affect the value of the ephippia Moina hatchery yields, which ranged from 10.67%-16.33% (Table 3).

Water quality parameters of Moina aquaculture at the beginning of culture include pH 7.7-7.5, temperature 27-31°C, hardness 53.09 mg/L and dissolved oxygen 5.5-5.7 mg/L. Dissolved oxygen concentration at the end of culture which ranges from 3.2-3.8 mg/L. The total ammonia at the end of Moina culture in treatments A and A4 is 0.25 mg/L (NH3/NH4) and other treatments are less than 0.25 mg/L (NH3/NH4) (Table 3).

| Parameter | Treatment | A | A1 | A2 | A3 | A4 |
|-----------|-----------|---|----|----|----|----|
| pH        | 7.7-7.5   | 7.7-7.5 | 7.7-7.5 | 7.7-7.5 | 7.7-7.5 |
| Temperature | 27-31 C | | | | |
| Dissolved oxygen (mg/L) | 5.6 - 3.4 | 5.5 - 3.5 | 5.6 - 3.6 | 5.5 – 3.8 | 5.6 – 3.2 |
| Hardness(mg/L CaCO3) | 53.09 | | | | |
| Total amonia (NH3/ NH4+) | 0.25 mg/L | <0.25 mg/L | <0.25 mg/L | <0.25 mg/L | 0.25 mg/L |
| Early culture | 0 mg/l | | | | |
| End culture | | | | | |

Notes: 1.10%, 2 20% and 3. 30% (decrease) and 4. 10% (increase)

3.2. Discussion

Different bran suspension feed concentrations in Moina culture from the fifth day to the twelfth day affected the population size. The reduction of the fifth-day bran suspension concentration from 0.45 mL/L to 0.36 mL/L (A2) caused a significant decrease in offspring production and was followed by the production of high male offspring. The quality and quantity of feed play an important role in Moina’s reproduction and growth. Decreasing feed concentration causes a decrease in fecundity and production of offspring [5, 14]. Feed concentration has direct implications for the availability of proteins, fats and amino acids, which affect the speed of development of eggs into offspring and the rate of early growth of offspring [6, 7].

The bran suspension contains protein (0.83%) and fat (0.79%) and amino acids arginine (0.05%) and histidine (0.02%) [11]. Arginine affects the regulation of endocrine and reproductive functions [15], whereas histidine affects the synthesis of DNA and proteins [16]. Vitamin B (thiamin and pyridoxine) in bran also function to increase the production of Moina’s offspring [17]. The culture of Moina using bran suspension feed concentrations of 0.5-0.66 mL/L (A4) produced the highest population peak of 15,373 ind/L. The culture of Moina with a lower concentration of bran suspension results in a low population peak.

The production of offspring per female parent Moina on the fourth day was 8.32-8.59 people in the population of 5757 ind/L. The reduction in the fifth-day bran suspension concentration from 0.45 mL/L to 0.36 mL/L (A2) caused a decrease in offspring’s production to 2.22 ind/parent but followed by the production of high male offspring (13.48%). [18] states that the formation of male offspring in cladocera is influenced by environmental factors, namely population density, temperature, nutrient quality and accumulation of metabolic products. The results of this study are in line with the study of [4] who found that high population density not only reduced offspring production per parent, but also was a major factor that could induce male production. The male production of M. macrocopa
*americana* increased when high population density followed by a sudden decline in the amount of feed available [8]. The feed is an important factor in the transition of parthenogenetic reproduction to sexual reproduction in cladocera, because the greatest energy produced by metabolism (68%) is used for reproduction [19].

Production of cladocera male offspring is also influenced by parent experiences including the availability of feed [10]. The culture of *Moina* using bran suspension feed with a concentration of 0.36-0.48 mL/L (A2) on the fifth day to twelfth produced the highest total production of male offspring 818±35 ind/L. Culture of *Moina* with lower bran suspension feed 0.32-0.42 mL/L (A3) produced the lowest total production of male offspring 292±48 ind/L. The concentration of bran suspension affected *Moina*'s reserve and energy status which affected its sensitivity to stress pressure due to a high population [9].

*Moina* females that reproduce parthenogenetically will not produce ephippia because *Moina* cannot make the transition from asexual reproduction to direct sexual reproduction [20]. Ephippia is only carried out by female offspring who do not reproduce parthenogenically [21], if cultivated with high density and sufficient feed availability [14]. The strategy to produce ephippia *Moina* is thought to be the same as daphnia, which will only producing ephippia if there are inducing factors (high population density) and sufficient PUFAS/EPA fatty acids are available. The synthesis of eggs ephippia *Moina* requires EPA (2.4 µg/mg dry weight) which is higher than for synthesizing substanous eggs (0.01 µg/mg dry weight) [22].

*Moina* Culture using bran suspension feed on the fifth to twelfth day as much as 0.5-0.66 mL/L (A4) produced the highest ephippia production of 410±20 grains/L and bran suspension concentration of 0.32-0.42 mL/L (A3) produces the lowest ephippia production of 122±3 grains/L. In copepod species, egg production is related to the availability of EPA fatty acids (20: 5n-3) in food and EPA fatty acid stores from the parent [23]. Bran suspensions contain high fat (10.62%) but with a concentration of linoleic α acid (0.20-0.27%) and low derivatives [24]. A linoleic acid and α linolenic acid which are essential fatty acids for cladocera [25] and some cladocera species are reported to be able to convert linolenic α acids to DHA and EPA with varying capabilities [26].

The EPA concentration also influenced male sexual activity, including the length of copulation time which directly affected the probability of fertilization [3]. [27] stated that eggs (ephippium) *Moina* if not fertilized would be released as ephippia without eggs or damaged. Based on this, the quality of ephippia can be determined from the number of ephippia containing two eggs (perfectly fertilized eggs). The number of ephippia containing two eggs besides being affected by the number of males is also determined by the number of sexual females. The low percentage of sexual females will cause males to spend more time chasing and intercourse with sexual females [28]. The results of this study also showed that the production of high male offspring could increase the production of ephippia containing two eggs to 89% in treatment A2.

*Moina* Ephippia with bran suspension feed has a hatching rate of 10.67-16.33%. The low hatching rate of ephippia *Moina* with bran suspension feed, due to the low concentration of linoleic α acid (0.20-0.27%) and its derivatives [24]. Which affects the amount of ephippia production and the value of hatchability, [22, 29]. [3] also found low levels of ephippia *D. magna* hatching from *Chlorella vulgaris* (low EPA concentration) of 28%.

Hatchability of ephippia is also influenced by several factors, including temperature, pH, and storage time [30], light intensity [31]. The cumulative hatching response of ephippia *Daphnia* and *Moina* has been shown to increase with increasing storage time (two to six months) [32]. In this study, ephippia *Moina* was stored at 5±1°C for 2 months to increase sensitivity during light exposure hatching (1,800 lux) according to the method of [13].

4. Conclusion

Culture of *Moina* using bran suspension feed with a concentration of 0.36-0.48 mL/L on the fifth day to produce the lowest production of offspring but produced the highest total production of male...
offspring. Increased production of male offspring increases the production of ephippia containing two eggs but does not increase the degree of hatching.

5. References
[1] Hiruta C and Tochinai S 2014 J. Morphology 275, 769-67
[2] Mubarak A S , Jusadi D, Zairin Jr M, Suprayudi M A 2017 AACL Bioflux 10,
[3] Choi J, Kim S, Hwan L G, Chang K, Kim D, Jeon K, Park M, Joo J, Kim H and Jeong K Ecol. Evol. 6, 2.817-2.832
[4] Azzurdi O, Yusoff F, Shamsudin M, Raha R, Alekseev V and Matias M 2012 Aquaculture 412-413, 131-135
[5] Hakima B, Khémissa C and Boudjéma S 2013 J. Biol. Sci. 5, 25-31
[6] Fink P, Pflitsch C and Marin K 2011 PLOS One Org. 6,
[7] Koch U, Creuzburg D, Grossart P and Stralie D 2011 Oecologia 167, 981-989
[8] D’Abramo L 1980 Limnol. Ocean. 25, 422-429
[9] Smolders R, Bailleul M and Blust R 2005 Aquat. Toxicol. 73, 155-170
[10] Mikulski A and Pijanowska J 2009 Fundamen. Appl. Limnol. Archiv Für Hydrobiol. 174, 301-305
[11] Mubarak A S, Jusadi D, Zairin Jr M and Suprayudi M A 2017 J. Akuakultur Indonesia 16, 223-233
[12] Delbare D and Dhert P 1996 FAO Fish. Technical Paper 361, 295
[13] Haghparast S, Shabani A, Shabanpour B and Hoseini S A 2012 J. Agric. Sci. Technol. 14, 811-820
[14] Zadereev E and Lopatina T 2007 Aquat. Ecol. 41, 255-261
[15] Jobgen W S, Fried S K, Fu W J, Meininguer C J and Wu G 2006 J. Nutri. Biochem. 17, 571-588
[16] Li P, Mai K, Trushenski J and Guoyao 2008 Amino Acid 37, 43-53
[17] Mehdipour N, Fallahi M, Takami G A, Vossoughi G and Mashincharian A 2011 Iranian J. Sci. Technol. 2, 157-163
[18] Smirnov N N 2014 Elsevier 129-149,
[19] Richman S 1958 Ecol. Monographs 2, 273-291
[20] Dodson S, Caceres C and Rogers C 2010 Academic Press 775-827
[21] Alekseev V, Stasio D and Gilbert J 2007 Springer Science & Business Media 1-214.
[22] Abrusan G, Fink P and Lampert W 2007 J. Limnol. Oceanography 52, 1724-1728
[23] Jónasdóttir S H, Visser1 AW and Jespersen C 2009 Marine Ecol. Progress 382, 139-150
[24] Faria S, Bassinelllo P and Penteado M 2012 Brazilian J. Pharmaceutical Sci. 48, 35-45
[25] Persson J and Vrede T Freshwater Biol. 51, 887–900
[26] Masclaux H, Bec A, Kainz M K, Perrie F, Desvilleletts C and Bourdier G 2012 Freshwater Biol. 57, 696-703
[27] Conde J, Valdés F, Romo S and Pérez C J. Limnology 70, 69-75
[28] Winsor G and Innes D 2002 Freshwater Biol. 47, 441-450
[29] Sperfeld E and Wacker A 2011 Freshwater Biol. 12, 1365-8427
[30] Stross R, 1966 Ecology 47, 368-374
[31] Pinceel T, Vanschoenwinkel B, Uten J and Brendonck B 2013 Freshwater Sci. 32, 517-524
[32] Jaime Y F 2009 Thesis School of Agriculture Food dan Wine (Adelaide: The University of Adelaide)

Acknowledgments
The authors are grateful to the Government of the Republic of Indonesia for all support to this research.