Manipulation the protein concentration in rice bran suspension toward fecundity and production of offspring \textit{M. macrocopa}.

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\textbf{Abstract}: The need of \textit{M. macrocopa} as a natural food can be fulfilled the quality and feed used optimally to support population growth. Protein is a nutrient that directly influences the fecundity and children of \textit{M. macrocopa}. This study aims to was the effect of different protein concentrations in the suspension of suspension on the fecundity and production of offspring \textit{M. macrocopa}. This study complete(CRD) consisting of 5 preparations of 4 replications (P0) protein 16.7% (P1) protein 23.75%, (P2) protein 30.80%, (P3) protein 37.85% (P4) protein 44.9%. The parameters used are the fecundity and production of offspring \textit{M. macrocopa}. \textit{M. macrocopa} culture was do with density of 20 ind/L water and feed changes are carried out every day. The results of this study showed that the different protein concentrations in rice bran suspension had a significantly different effect (p <0.05) on the fecundity and production of offspring \textit{M. macrocopa}. that produced the highest fecundity and offspring at a protein concentration of 44.9%, as much as 30.25 ± 0.95 items/parent and child production per parent as much as 28.5 ± 0.57 ind/parent.

1. Introduction
\textit{M. macrocopa} is a potential natural food for freshwater fish seeds, due to its high nutritional value, easy to digest and has high reproductive power, fast breeding and easy to develop and has a size that corresponds to the mouth of the fish \cite{1, 2, 3, 4}. Problems in the use of \textit{M. macrocopa} as an unconstrained natural feed on the availability of \textit{M. macrocopa} both from nature and from culture. Population density in \textit{M. macrocopa} culture varies because of the feed used by different qualities (proteins). \textit{M. macrocopa} needs as natural feed can be fulfilled when the quantity and quality of feed used optimally support population growth \cite{5, 6}. According to \cite{7} the quantity and quality of feed is one of the important factors in the culture of \textit{M. macrocopa} which directly affects the growth of the population.

Protein is one of the nutritional factors affecting the growth of the population that can be seen from the fecundity, development of embryo and children \textit{M. macrocopa}. Amino acids, protein concentration, fats in the feed affects the production of offspring and the speed at which eggs develop into young \cite{8}. Information on optimal protein concentrations in the culture of \textit{M. macrocopa} which
produces the fecundity and highest production of saplings is not yet available. It is therefore necessary to optimize the protein concentration to produce a uniform population in fulfilling the culture needs.

Culture of *M. macrocopa* using rice bran feed containing protein (11-13%) [9], produced a child as much as 8-12 ind/parent [10]. Culture of *M. macrocopa* can produce a higher child by using Chlorella spp. feed which contains 51-58% protein [11] and has the highest fecundity of 37 eggs/mother [12].

Increased protein rice bran can be done by the addition of flour ISP (*Isolated soy protein*), because flour ISP (*Isolated soy protein*) has a protein content (90%) [13]. Based on the explanation above, it can be concluded that the protein concentration adjustment of rice bran should be done as it will affect and increase the fecundity and offspring production *M. macrocopa*. The purpose of this research is to determine the influence of different protein concentrations in the rice bran suspension against fecundity and offspring production *M. macrocopa*. The results of the study will greatly help increase the population in the culture of *M. macrocopa*.

2. Materials and methods

2.1. Materials

This study used *M. macrocopa* obtained from the hatching of epiphia, rice bran and ISP (*Isolated soy protein*), sterilized water with dolomite (CaMg(CO$_3$)$_2$).

2.2. Place and time of research

This research was conducted in April 2020 at the Laboratory Anatomy and Culture, Faculty of Fisheries and Marine UNAIR Surabaya.

2.3. Research design

This study used a completely randomized design (CRD) with experimental methods, five treatments and each treatment was repeated four times namely control protein 16.7 % (rice bran 100 gr), P1: protein 23.75 % (rice bran 93 gr + flour isolated soy protein 7 gr), P2: protein 30.80 % (rice bran 86 gr + flour isolated soy protein 14 gram), P3: protein 37.85 % (rice bran 79 gr + flour isolated soy protein 21 gr) dan P4: protein 44.9 % (rice bran 72 gr + flour isolated soy protein 28 gr).

2.4. Method

2.4.1. Inoculant preparation

The provision of inoculant is carried out by means of hatching epiphia. Epiphia was hatched first to obtain offspring of *M. macrocopa* (female partenogenesis) which was used as a treatment in the study. Epiphia is based in a container measuring 300 ml. The first hatching lasts for 30 hours, dark green Epiphia[14] and the epiphia that have been transparent white hatched are separated into other containers. After 24 hours, the developed child (female partenogenesis) *M. macrocopa* was transferred to another container to be selected to obtain a descendant derived from one parent. The parent that produces the most child will be used. The resulting offspring of *M. macrocopa* are cultured with a stocking density of 20 ind/L.

2.4.2. Water treatment

The culture media used in this study is derived from tandon owned by the Faculty of Fisheries and Marine Airlangga University. The water from the Tandon is inserted in the 1000 L fiber body and then added dolomite lime (CaMg(CO$_3$)$_2$) as much as 2100 grams/ton so that the consciousness of > 40 mg/L and aerated at least three days before use. Water from the fibre tub is filtered with nylon measuring 40 μm before being inserted into the treatment container to remove the zooplankton and competitors.

2.4.3. Making rice bran Suspension

The preparation of rice bran suspension and *isolated soy protein* suspension is done by preparing rice bran, isolated soy protein, and water. For (P0) of the rice bran used as much as 100 g and water as much as 500 ml are put in a blender, homogenized using a blender at a speed of 2000 rpm until well
blended for ± 5 minutes 2 times. Than, the water suspension was filtered using a filter with sizes of 2 mm, 0.1 mm and 40 μm. The suspension passes plus the volume to 500 ml [15].

2.4.4. *M. macrocopa culture*

The culture of *M. macrocopa* was carried out for seven days in a container of jars containing fresh water as much as 300 ml and was aerated. *M. macrocopa* density in each jar is as much as 20 ind/L, using the child of the culture of 20 ind/L density and aged less than 24 hours. Water change is done every day after that the feeding is done according to the treatment. Feed is given once a day in the morning at 10.00 WIB. During maintenance carried out measurements of water quality which includes DO, temperature and pH.

2.4.5. *Research parameters*

The parameters in the study were calculating the *fecundity* and production of offspring *M. macrocopa*. The fecundity observation was conducted by sampling 4 *M. macrocopa* tails, observed using the aid of 100x magnification microscope and repeated observations 2 times. According to [15], the production offspring per female/parent *M. macrocopa* was calculated using the following formula:

\[
\text{Offspring production per broodstock} = \frac{\text{total offspring produced}}{\text{total broodstock}}
\]

2.5. *Data analysis*

The research data obtained was analyzed using Analysis Of Variance (ANOVA), when there was a difference followed by duncans multiple tests with a real level of 5% [16].

3. *Result and discussion*

3.1. *Result*

3.1.1. *Fecundity*

Result of fecundity *M. macrocopa* cultivated using different protein concentrations with rice bran suspension displayed in table 1.

| No | Protein concentrations in feed | Fecundity          |
|----|--------------------------------|--------------------|
|    |                                | First birth | Second birth | Third birth |
| 1  | 16.7%                          | 18±0.81d     | 19±0.81c     | 21.5±1e    |
| 2  | 23.75%                         | 20.5±0.57e   | 22.25±0.5d   | 23.75±0.5d |
| 3  | 30.80%                         | 22.75±0.95b  | 24.25±0.5c   | 26.25±0.5c |
| 4  | 37.85%                         | 23.75±0.95b  | 26.00±0.81b  | 28.75±0.5b |
| 5  | 44.9%                          | 25.75±0.5a   | 27.75±0.95b  | 30.25±0.95a |

Note: Different superscript in the same column indicates a significant difference (p<0.05)

The results of the ANOVA test showed that the culture of *M. macrocopa* using a rice bran suspension feed with a different protein concentration gives a distinct effect of real (P<0.05) to the fecundity *M. macrocopa*. Fecundity *M. macrocopa* highest in the age of 1, 2, and 3 in the culture of using a suspension of feed with a protein concentration 44.9% as much as 25.75 ± 0.5 grains/parent, 27.75 ± 0.95 grains/parent, and 30.25 ± 0.95 grains/parent.

3.1.2. *Production of offspring on each broodstock*

Result of production on each broodstock *M. macrocopa* cultivated using different protein concentrations with rice bran suspension displayed in table 2.
Table 2. Production of offspring on each broodstock

| No | Protein concentrations in feed | Production of offspring on each broodstock |
|----|--------------------------------|-------------------------------------------|
|    |                                | First birth | Second birth | Third birth |
| 1  | 16,7 %                         | 14,25±0,5e | 15,25±0,5e | 16,75±0,5f |
| 2  | 23,75 %                        | 18,5±0,57d | 20,75±0,95d | 22,75±0,5f |
| 3  | 30,80 %                        | 20,75±0,95c | 22,5±0,57c | 25,5±0,57b |
| 4  | 37,85 %                        | 21,75±0,5b | 24,0±0,81b | 26,25±0,5b |
| 5  | 44,9 %                         | 23,5±0,57a | 25,75±0,95a | 28,5±0,57a |

Note: Different superscript in the same column indicates a significant difference (p<0.05)

The ANOVA test results showed that *M. macrocopa* culture used rice bran suspension feed with different protein concentrations against production of offspring on each broodstock *M. macrocopa* has a significantly different effect (p<0.05). Production of offspring on each broodstock highest births 1, 2 and 3 in culture using rice bran suspension feed with a protein concentration of 44.9% as much as 23.5 ± 0.57 ind/parent, 25.75 ± 0.95 ind/parent, 28.5 ± 0.57 ind/parent consecutively.

![Figure 1. Average fecundity and offspring production *M. macrocopa*.](image)

Based on the results obtained from the average Fecundity and offspring production of *M. macrocopa* cultivated using a different protein concentration with Rice bran suspension there is a difference. In the culture of feed suspension with a protein concentration of 44.9% produce high fecundity, which is classified into 1, 2, and 3 consecutive as much as 25.75 ± 0.5 grains/parent, 27.75 ± 0.95 grains/parent, and 30.25 ± 0.95 grains/parent. And the result of the child's production is classified into 1, 2, and 3 as much as 23.5 ± 0.57 ind/parent, 25.75 ± 0.95 ind/parent, 28.5 ± 0.57 ind/parent in a coordination. While the lowest fecundity is being classified into 1, 2 and 3 in the culture of using a suspension feed rice bran with a protein concentration of 16.7% as much as 18 ± 0.81 grains/parent, 19 ± 0.81 grains/parent and 21.5 ± 1 grains/parent and offspring production in the culture of using a suspension feed rice bran with a protein concentration 16.7% as much as 14.25 ± 0.5 ind/parent, 15.25 ± 0.5 ind.

3.1.3. Water quality
Measurement of water quality is done to know the environmental condition and sustainability of the culture *M. macrocopa*. Use suspension feed rice bran with different protein concentrations. Water quality is observed as temperature, (dissolved oxygen) DO, pH, and ammonia. The measurements are done in the morning at 10.00 WIB. The condition of air quality *M. macrocopa* growth is at a
temperature range of 26-31 °C, DO 3-5 ppm and ammonia ranges from < 2 (mg/L) and pH ranges from 7, 8 – 8, 3.

**Table 3. M. macrocopa water quality**

| Parameters   | 16.7 % | 23.75 % | 30.80 % | 37.85 % | 44.9 % |
|--------------|--------|---------|---------|---------|--------|
| Temperature (°C) | 26.6 - 28.3 | 26.7 - 28 | 27.8 - 28.6 | 27.4 - 29.2 | 28.2 - 30.1 |
| pH           | 6.9 - 7.5 | 6.9 - 7.7 | 7 - 7.7 | 7.1 - 8 | 7 – 8 |
| DO (mg/L)    | 3.13 - 4.48 | 3.40 - 4.74 | 3.22 - 4.85 | 3.43 - 5.15 | 3.33 - 4.97 |
| Ammonia (mg/L) | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |

3.2 Discussion

Increased protein concentration in the feed suspension as the feed of M. macrocopa affects the fecundity and offspring products, but has no effect on the level of livelihood of the parent M. macrocopa. The quantity and quality of feed is an important factors in the culture of M. macrocopa which directly affects population growth [7].

Protein is the primary source of nutrients needed in both quality and quantity feed, proteins play a role in the formation of materials for the growth of living beings, and are also important for producing enzymes and other substances [17]. The high concentrations of protein, amino acids and fats in in feed may increase the reproductive of the Cladosera parthenogenesis, increased fecundity, speed of development embryos and offspring production on each broodstock [8].

Increased protein concentration in the suspension feed rice bran can improve the fecundity M. macrocopa, where the highest fecundity in the culture of M. macrocopa by using a suspension of rice bran feed containing 44.9% protein, as much as 30.25 ± 0.95 grain/parent.

The lowest M. macrocopa fecundity in the culture of rice bran suspension feed containing 16.7% protein as much as 21.5 ± 1 grain/parent. Fecundity is influenced by the process of developing eggs in the pamping bag M. macrocopa. The egg development process can be influenced by water quality, quantity and quality of feed. The high protein and fat content can accelerate embryonic development in cladocera [8]. The successful development of the embryo in the round sac of M. macrocopa is very important in the success of the production of offspring. Fecundity kladocera is influenced by several factors such as proteins, fats and amino acids (especially arginine and histidine) [18].

The increase in protein concentration in rice bran suspension feed can increase the total production of offspring M. macrocopa, where the total offspring production of the highest in the culture of M. macrocopa by using a suspension of rice bran feed containing 44.9% protein 199.75 ± 4.64 Ind/L. Total production of M. macrocopa is the lowest in the culture of rice bran suspension containing 16.7% protein as much as 114 ± 7.11 Ind/L. Total increase in offspring production can be caused by the increase in body size of the female parent, the larger size of the parent resulting in greater depleted sac, so that more embryos develop in the seam [19].

The production of offspring by the highest parent in the culture using a suspension feed rice bran with a protein concentration of 44.9% as much as 28.5 ± 0.57 ind/parent. Increased protein concentration leads to an increased availability of feed nutrients in culture media. Protein is one of the nutrients that play an important role in maintaining the functioning of damaged tissues and the formation of new tissues, so that proteins affect the growth of larvae [20]. The proteins contain the amino acids arginine and histidine. Increased arginine and histidine can affect the fecundity [21]. The amino acid arginine affects the setting of endocrine and reproductive functions [22], whereas histidine affects the synthesis of DNA and proteins [23].

Cultivating a suspension of rice bran feed with different protein concentrations resulted in the number production of offspring per parent, different from the amount of fecundity. The difference in production of offspring value per parent and fecundity is influenced by the development process of M. macrocopa's dreary bag. According to [19], the larger size of the parent caused the curling sac to be larger, so more embryos were developed in the curling pockets. The egg development process can be
influenced by the quality and quantity of feed and water quality. According to [7], the quality and quantity of feed is one of the important factors in the culture of *M. macrocopa* which directly affects the growth of the population.

Increased protein concentrations affect the amino acids FAA (free amino acid) increases. The FAA (free amino acid) is a residual result of protein synthesis, the FAA (free amino acid) serves as a source of energy and protein. FAA (free amino acid) that affects the growth and development of embryos and will increase the number of eggs that develop into children [24]. The content of the FAA will make the previous oocytes of ovulation and spawning [25]. Therefore the value of the fecundity and production of offspring is different, as well as increased protein concentrations affects the occurrence of a shorter distance between the value of fecundity and the production of offspring *M. macrocopa*. Embryos that do not develop into a child will die and be reabsorbed by *M. macrocopa* parent which will be used as nutrient for *M. macrocopa* body.

Water quality is another factor that affects culture using a rice bran suspension feed with different protein concentrations such as pH, temperature, DO (dissolved oxygen) and ammonia. According to [26], that the environment that supports the growth of *M. macrocopa* is in temperature in between of 26-31 °C, DO 3-5 ppm and Ammoniac ranges from < 2 mg/L and pH ranges from 7.8 – 8.3 [27].

4. Conclusion
The cultivation of *M. macrocopa* with rice bran suspension feed with different protein concentrations affected the fecundity and production of the offspring of *M. macrocopa*. That produces the highest fecundity and offspring at a protein concentration of 44.9%, as many as 30.25 ± 0.95 grains / parent and production of offspring per parent as many as 28.5 ± 0.57 ind / parent as many as 28.5 ± 0.57 ind / parent.

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