**Reproductive Character of the Ricefield Eel (*Monopterus albus* Zuieuw) in Babakan Village, Karang Lewas District, Banyumas, Central Java**

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**Abstract.** The ricefield eel (*Monopterus albus* Zuieuw) is a protogynous hermaphrodite. One of the villages in Banyumas Regency, which provides a high potential habitat for ricefield eel, is Babakan, but information about their reproduction cycles untill now is still lacking. The aims of this research were to understand the reproductive characters were Gonad Maturity Level (GML), Gonad Maturity Index (GMI), fecundity, egg diameter and to understand the correlation of body length with both GML and GMI of ricefield eel. The method used was survey with purposive sampling design. The sampling was done once in a month and four times during the period of November 2017 to March 2018. The samples were 30 in each sampling, with total sample of 120 eels. The results showed that the ricefield eels experience intersex phase at 30.5-30.7 cm of body length. The ricefield eel less than 30.5 cm was a female and the ricefield eel more than 30.7 cm was a male. The sex of ricefield eel based on stages of gonad development were GML I (there were oocytes equipped with nucleus and cytoplasm), GML II (the presence of cortical alveoli stage), GML III (the stage of vitelogenesis), and GML IV (mature stage). Intersex stage was characterized by the presence of degenerated oocytes and the presence of testicular lobes. The male stage was characterized by a testicular lobe. The dominant GML in November, December, January, and March were GML I and II, GML I and II, GML III, and GML IV, respectively. Fecundity of the ricefield eels during the research was between 207 and 370 eggs, with an average of 252 eggs. The egg diameter of 0.22-2.5 mm was indicating that the ricefield eel was on the batch spawner. The highest GMI of ricefield eels was occurred in January and the highest GMI was within the interval of 00017-0.0242 during the entire month of sampling. The body length of the ricefield eel was not correlated with the GML. On the other hand, the body length of the eel was correlated with the GMI.

**Key words:** ricefield eel (*M. albus* Zuieuw), gonad maturity index (GMI), fecundity, gonad maturity level (GML), oocytes.

1. **Introduction**

Ricefield eel (*Monopterus albus* Zuiew) is included in *Synbranchiformes* order. It is a protogynous hermaphrodite, which has an ability to change its sex. It has a female sex on its young age, which will be changed into male on its old age, after undergoes intersex phase [1]. Ricefield eel can be consumed as the main source of protein. The proteins from ricefield eel contain several essential amino acids, such as lysine and methionine. Ricefield eel also can be used as the mixture of chicken food [2].

The demand of ricefield eel by the society for daily consumption is quite high. The data obtained from Fish Quarantine Station, Quality Control, and First Class Fish Security Office in Palu showed that during 2016, there were 624,050 of ricefield eel sold to Denpasar City for 58 times [3]. The demand of ricefield eel in Asia was 50-60 tons per day. However, 80% of the demand could not be fulfilled yet [4].

The central of ricefield eel production in Indonesia was only Yogyakarta and West Java [3]. High gap between demand and the production, provide an opportunity for other provinces to improve their ricefield eel production. Banyumas Regency can become a potential eel producer in Central Java because it has (32,528 hectare) [5].

One of the villages in Banyumas Regency, which has a high potential ricefield area for the habitat of ricefield eel is Babakan village. Babakan Village is located in the mountain area south of Slamet Mountain. The extent of this village is 301 hectare, which consists of 88.9 hectare of the rice filed area, 77 hectare of the settlement area, 118.8 of the yard area, and 16.3 hectare of the public facilities area [6]. The people of Babakan were unable to cultivate the ricefield eel, because of no information regarding its reproductive characteristics.

Another problem was the difficulty in obtaining the parents type of ricefield eel and its seeds. All this time, manual searching and capturing of the ricefield eel were the only way to fulfil the market demand. If these problems were left unsolved, the ricefield eel on the nature will be decreased. The dependency toward ricefield eel capturing method can be solved by understanding its reproductive characteristics [7].

Reproductive characteristic is the most important thing to be understood in order to cultivate the species of aquaculture. The knowledge about reproductive character of the fish is a strategy to ensure the life of its descendants [8]. Reproductive character can be observed macroscopically (by observing its morphology) and microscopically (by observing its histology) [9]. The observation of reproductive characteristics according to [10] were including body weight, body length, fecundity, egg diameter, and spawning pattern. In addition, the important aspect of reproductive characteristic was the gonad maturity, which can be measured qualitatively as Gonad Maturity Level (GML) and quantitatively as Gonad Maturity Index (GMI) [11].

The aims of this research were to understand the reproductive character of ricefield eel (the sex was based on body length and stages of gonad development along with GML, GMI, fecundity, and egg diameter) and to understand the correlation of body length with both GML and GMI of the ricefield eel in Babakan Village, Karang Lewas, Banyumas.

1. **Method**
	1. *Research Material.*

Materials used in this research were the ricefield eel, either male or female; water; 10% of NBF; 70%, 80%, 90%, and 96% of absolute alcohol; xylol solution, aquadest, Hematoxylin-Eosin stain, entellan, paraffin, gelatin, and tissue paper. Tools used in this research were a set of surgical tools, millimeter block, sample bottle, bucket, label paper, preparation basin, aluminium foil, beaker glass, measuring cup, electric stove, pan, microtome, injection spuit, brush, camera, stopwatch, object glass, cover glass, microscope, micrometer, basin, incubator, staining jar, sitting lamp, and analytical scale.

The amount of subjects in this research was 120 eels (30 eels obtained from each sampling). The sampling was performed on November 2017, December 2017, January 2018, and March 2018, in Babakan Village, Karang Lewas Sub-district, Banyumas. The obtained ricefield eel was classified into 8 class of body length interval as follows: 23-25.2, 25.3-27.5, 27.6-29.8, 29.9- 32.1, 32.3-34.5, 34.6-36.8, 36.9-39.1, and 39.2-41.4 cm.

* 1. *Research Location and Time.*

This research was performed in Babakan Village, in the south of Karang Gandul Station, Karang Lewas Banyumas. The observation was performed in the Laboratory of Animal Structure and Development, Faculty of Biology, Jenderal Soedirman University, Purwokerto. The observation also took place in Trial Station of PSDP D3 of Jenderal Soedirman University. This research was performed between November 2017 and April 2018.

* 1. *Research Design.*

Current research was using survey with purposive sampling design. The sampling was performed in 4 different months, once in a month, and 30 eel for each sampling. The total amount of sample was 120 eel. The capturing activity was performed using hands in the morning.

The parameter observed in this research was the reproductive characteristic of ricefield eel. The supportive parameters in current research were temperature and pH. The variables measured in this research were ricefield eel’s gonad morphology, body length, body weight, fecundity (amount of eggs), diameter of egg, GML, GMI, and gonad histology.

* 1. *Research Protocol*
		1. *Body Length and Weight Measurement.* Measurement of body length and weight were performed after the ricefield eel was killed. The measurement of body length was performed by measuring the length from the top of the head to the end of the tail by using a millimeter block. The measurement of body weight was performed using analytic scale with 0.01 g of accuracy [12].
		2. *Fecundity.* Total fecundity was counted using amount method. Amount method was used because of the egg had a relatively large size. Therefore, the result will be more accurate if all the eggs were counted. The fecundity counting was performed on the eggs, which had reached GML III and IV. The eggs were obtained from the gonad of the female ricefield eel. They were released from the membrane, which was surrounding them. Furthermore, they were counted one by one. This method was quite accurate to count the little amount of fish egg [11].
		3. *Measurement of Egg’s Diameter.* Measurement of egg’s diameter was performed under the light microscope and using the calibrated ocular micrometer. The measurement was performed on eggs with different GML (GML III and IV). The measurement was performed by taking 50 eggs and the diameter was calculated using Effendie’s equation [10]. The equation is: Diameter of the ovum = the ocular scale x calibration number. The value of 1 on the objective scale is as follows: 1 ocular = (objective value/ocular value) x objective scale.
		4. *Gonad Maturity Index (GMI).* Gonad Maturity Index (GMI) was calculated by comparing gonad’s weight with fish’s body weight and multiplied by one hundred percent [11].
		5. *Gonad Maturity Level (GML).* Gonad Maturity Level (GML) can be observed morphologically and histologically.

Table 1. Characteristics of Gonad maturity Level [13]

|  |  |
| --- | --- |
| GML | Characteristics |
| I | The eggs could not be seen visually and the proportion of the eggs was more than the proportion of male eel. |
| II | The eggs could be seen visually, small and the proportion was 80-90% of the gonad. |
| III | The eggs could be seen clearly by visual, large, adhered to each other, and the proportion of the eggs was 95% of the gonad. |
| IV | The eggs could be seen clearly by visual, large, adhered to each other, and the proportion of the eggs was nearly 100% of the gonad. |
| *Intersex* | The proportion of sperm and egg was equal. |

* + 1. *Preparation and observation of Gonad’s Histology.* tThe gonad was observed micro-morphologically by histology observation using modification of paraffin method [14]. First, the ricefield eel was dissected, followed by take its gonad. Furthermore, the gonad was fixed by an NBF solution for 24 hour The steps of making histological preparation by paraffin method were washing, dehydration, dealcoholization (clearing), and infiltration on a paraffin, embedding on a paraffin block, sectioning, affixing on the gelatinized object glass, deparaffinization, staining, mounting, labelling, and microscope examination, respectively.
	1. *Data Analysis*

The data of GML, GMI, fecundity, and diameter of the ricefield eel either by histological measurement, were analyzed descriptively and qualitatively. The correlation of body length with GML and body length with GMI, were analyzed using SPSS 16.0. The determination of sex was performed according to the body length and was analyzed descriptively.

1. **Result and Discussion**

Captured eel in November reached the highest frequency within the interval of 27.6-29.8 cm of body length. The same interval of body length was shown by the majority of ricefield eels, which were captured in December. On January, the number of captured eels reached maximum amount on the interval of 29.9-32.1 cm of body length. The highest number of ricefield eel on March was also had the same class of body length interval as January.

Generally, the result of this research showed that ricefield eels, which underwent intersex phase, were within the body length interval of 30.5-30.7 cm. Therefore, the eels with body length within the interval of 23-25.2, 25.3-27.5, and 27.6-29.8, were a female. The eels with the body length interval within 29.9-32.1 cm, were an intersex and a male.

The eels with body length within the interval of 32.3-34.5, 34.6-36.8, and 36.9-39.1, were a male. Bonvenuto C, *et al*., [15] stated that the transformation of gonadal structure from female to male through an intersex phase were approximately need 2 to 4 months of duration.

The number of captured male eel from November until December 2017 was shown to be slowly reduced. The number of captured female eel was more than the number of captured male eel. This condition was occurred due to an imbalance of the number of female and male in hermaphrodite fish [16]. In addition, this finding also similar with the research by [15].

Figure 1. The amount of captured eels and body length interval

Figure 2. Gonad Maturity Level on November

**Figure 3. Gonad Maturity Level on December**

Figure 4. Gonad Maturity Level on December

Figure 5. Gonad Maturity Level on January

Figure 6. Gonad Maturity Level on March

Gonad maturity level on November have presented on Figure 2. The majority of captured ricefield eel on this month was on GML II, which was at body length interval of 26-28.3, 28.4-30.7, 30.8-33.1, and 33.2-35.5 cm. We found an intersex at the body length interval of 28.4-30.7 cm. This eel had 30.7 cm of body length. The GML III and IV were only found at the body length interval of 30.8-33.1 cm and 38-40.3 cm.

It can be seen from Figure 3 that GML I and II of captured ricefield eel on December were at the body length interval of 23.2-26, 26.1-28.9, and 29-31.8. The intersex was found at the body length interval of 29-31.8 cm. The GML III and IV were not found much. The captured ricefield eel on GML III dominated the findings on January. It was found at the body length interval of 23-25, 25.1-27, 27.2-29, 29.3-31, and 33.5-35.5 cm. The ricefield eel on GML IV was found at the body length interval of 23-25, 25.1-27, 27.2-29, and 29.3-31 cm. The male eel was only found at the body length interval of 31.4-33.4 and 33.5-35.5 cm (Figure 5).

The captured eel on March showed gonad development from the GML I (the young female) to GML IV (adult female) and a male. This is illustrated on Figure 6, which showed that GML IV was at the body length interval of 25.9-28.4, 28.5-3, and 31.1-33.6 cm. The amount of GML IV on this month was higher than its amount on the several months before. There was no intersex eel on this month. The eel with body length within the interval of 33.7-36.2 cm was also not found. The amount of ricefield eel on a male phase in this month was the highest among all month. The male was found within the body length interval of 28.5-31, 31.1-33.6, and 36.3-38.8 cm.

Generally, the captured ricefield eels in Babakan Village, Karang Lewas, Banyumas showed an annual spawner pattern. It was shown from the GML of the captured eels. On November and December, the GML I and II started to develop and dominated the finding. On January, GML III and IV dominated the finding. The GML IV was found more on March. The highest number of male eel also found on March. This finding was similar with [17], who stated that ricefield eel was an annual spawner.

The GML I, II, III, and IV showed that the ricefield eel was on a female phase, while an intersex was the transition between a female and a male eel. A male eel also found in this research. The captured eels on November with body length interval of 28.4-30.7 were found to be at GML I, GML II, GML III, GML IV, and intersex phase. The GML I was only found at the body length interval of 26-28.3 cm and 28.4-30.7 cm. The transformation of ricefield eel into a male was found at the body length interval of 30.8-33.1 cm and 38-40.3 cm. The GML I, II, III, and IV of the captured eels on December was found at the body interval of 23.2-26 cm.

The GML I, II, and intersex were still found at the body length interval of 29-31.8 cm. The GML IV was also found at the body length interval 31.9-34.7 cm. The highest amount of male eel was at the body length interval of 31.9-33.4 cm and 37.7-40.5 cm.

The result of the sampling on January showed that the GML I was started to be found at the body length interval of 27.2-29.2 cm. It was also found at the body length interval of 31.4-33.4 cm, even though male eels were also found at this interval. This finding might be as the result of larger size of those eels. Therefore, the sex differentiation was not occurred yet, even in the same level of age [18]. This event also can be caused by the huge availability of nutrition, which boosted the growth of different ricefield eel [19]. The GML II and III were on the same body length interval, except for those at the body length interval of 31.4-33.4 cm. The body length intervals of eels with GML IV were at 23-25 cm and 29.3-31.3 cm. The intersex phase was found at the body length interval of 29.3-31.3 cm. At the body length interval of 33.5-35.5 cm, eels were found to be at the phase of GML II, III, and male. There was no an intersex among the captured eels on March. At the body length interval of 23.3-25.8 cm, there were eels with GML I and II. The eels with GML III were only found at 31.3-33.6 cm. The highest amount of male eels was found at the body length interval of 36.3-38.8 cm.

The result of the current research was different with [18] which stated that the changes of sex from a female to a male was occurred at the body length of 29 cm. In the current research, eel underwent an intersex phase at body length interval of 29.3-31.3 cm. There were several factors, which might contributed to these findings, such as nutrition [18], genetics, or environment [20].

The environmental factors, which were observed in this research were temperature and pH. The temperature observed in this research was 26 0C, while the pH was 6. These findings were similar with the previous research, which stated that the temperature of a ricefield eel was within the interval of 23-26 0C and the pH within the interval of 5.8-7 [21]. It also indicated that the environment of Babakan Village was favorable for the growth of ricefield eel.

According to the result of the current research, a female became an intersex within the body length interval of 30.5-30.7 cm. This finding was similar with the result of a research by [22] which stated that an intersex phase was occurred within the body length interval of 26-40 cm. The ricefield eel was a protogynous hermaphrodite, which can change its sex from a functional female to an intersex and a functional male during its life [23]. Intersex individual is marked by the development of an Ovarium to a testis. Male individual is marked by the development of testicular tissue. Intersex phase is occurred naturally. The cause of the occurrence of this phase was still poorly understood.

The interval of the GMI with the highest frequency of all month was within 0.0017-0.0242 (Figure 7). This data were illustrated the average of eel’s amount of all month of sampling, which had a low gonad weight, because it were still on the GML I and II. These findings were similar with the result of a research by [17], which stated that a ricefield eel on the GML I or II had a low gonad weight. [24] also found that the component of GMI was the gonad weight. Therefore, if the gonad weight is low, then the GMI will also low.

Gonad weight was started to have a mature sex on GML II with the GMI interval of 0.0469-0.0694 and body length interval of 25.3-27.5 cm. The captured ricefield eel on January had a mature gonad and had the highest GMI among all of the captured eels in other months. This might occurred because of the average of captured eel on January were at the GML III. The maximum value of GMI of the captured eels was 0.1822 with body length of 23.5 cm, and at the GML III. These findings were similar with the previous research, which stated that the highest GMI on a female ricefield eel can be obtained from an eel at the GML III, because of the gonad weight reached its maximum weight at GML III. It was the sign of egg release [25].

Fecundity obtained during the research from November to March was 270-370 eggs, with an average of 252 eggs (egg diameter 0.225-2.5 mm). The value of fecundity from current research was similar with the research conducted by [17], which revealed the fecundity between 38 and 625 eggs, with an average of 295 eggs. [24] also found that the average value of fecundity of the ricefield eel was 280 eggs. Ricefield eel was among the animal with low fecundity, which was within the value of 200-300 eggs [26]. It is because of the parental care. Ricefield eel is also included on the group of nest-breeding fish [27].

Figure 8 and 9 showed that the diameter of the eggs of ricefield eel had a different size. In every class of diameter interval, there were eel’s egg with a certain number on every month of sampling with GML III or IV. The lowest egg’s diameter was 0.2250 mm, while the highest egg’s diameter was 2.5 mm. These results was similar with the result of a research by [17], which stated that the development of ricefield eel’s eggs was not equal. There was an egg with the different level at the same Ovarium. This statement was also supported by [28]. Measurement of egg’s diameter showed that the ricefield eel was a batch spawner, because it had a long spawning season [17].

Figure 7. Gonad maturity Index of Ricefield Eel

Figure 8. Interval of Ricefield Eel’s Egg Diameter at GML III

Figure 9. Interval of Ricefield Eel’s Egg Diameter at GML IV



Table 2. Correlation of Body Length with the GML

The correlation of body length with GML was analyzed using SPSS 16.0 and Pearson correlation test with 5% of signification (Table 2). Analysis of body length with GML was including the GML I, II, II, and IV. The result of correlation analysis showed no correlation between body length and GML with p value >0.05. The result of correlation analysis on table 2 was supported by a research by [22], which revealed that there was only correlation between body length and sex and there was no correlation between body length and GML. The eel with small body had female sex, while a male had a larger body. Intersex phase can be identified by histological observation. However, there was no certain body length value that precisely defined the sex phase of eel. The body length of every region could be different. It was related to the environmental condition on every habitat of eel.

Correlation between eel’s body length and GMI was analyzed using SPSS 16.0 and Pearson correlation test with 5% of signification (Table 3). The result of correlation analysis showed significant correlation between body length and GML with p value <0.05. The value of r was 0.207. The negative sign on Pearson correlation indicated that longer body weight correlated with lower GMI.

This result was similar with the result of a research by [25], which stated that generally, the value of GMI was depend on the body length and GML. Furthermore, negative correlation between GMI and body length was because of the longer body length will produce higher GML and high GMI.

Spawning season [29] and will be low at an intersex phase. The value of GMI will be increased at the higher GML and the highest GMI will be obtained at GML IV. Gonad degeneration of Ovarium at intersex phase was related with testicular development and lower GMI [29].

Sex of ricefield eel cannot be determined only using its body length. The histology of its gonad must be observed to determine its sex. The development of gonad histology of the ricefield eel at GML I was marked by egg cell with nucleus and cytoplasm, while at GML II there were cortical alveoli, mucous, and cytoplasm. Cortical alveoli was a circle without color at the edge of oocyte. At GML II, there were an oocyte with larger diameter, central nucleus, increased number of cortical alveoli, yolk granule, and radiate zone. At GML IV, there were a mixed of cortical alveoli and yolk granule, which form a larger and the shift of the nucleus. Intersex phase was marked by a degenerated oocyte and the presence of testicular lobe. Male phase was marked by the development of testicular lobe. The description of this histology was similar with [26]. [19] also stated that GML I was a nucleolar and perinucleolar stage. The GML II was marked by cortical alveoli and yolk globe. The GML III was a late globular stage and the GML IV was a mature-ripe stage or follicle atretic stage.

1. **Conclusion**

According to the result of current research, can be concluded that the captured eel underwent intersex phase on the body length interval within 30.5-30.7 cm. The sex of ricefield eel according to the histologic feature of its gonad was GML 1 with oocyte, nucleus, and cytoplasm, GML II with alveoli cortical, GML III or vitelogenesis, and GML IV or mature phase. Intersex phase was marked by a degenerated oocyte and by the presence of testicular lobe. Male phase was marked by a testicular lobe. Fecundity of ricefield eel was 207 to 370 eggs with an average of 252 eggs. Diameter of the eggs was within the range of 0.225-2.5 mm and was included on a partial spawner. The highest GMI of ricefield eel was found on January. The highest GMI in all month of sampling was within the interval of 0.0017-0.0242. The body length of ricefield eel was not correlated with its GML. It was correlated with its GMI.

The possible topic for the next research is about the possibility of ricefield eel in increasing field productivity. The factors that affect the lives of ricefield eel, such as nutrient availability and sex ratio also can be the topic for further investigation. Therefore, the cultivation of ricefield eel can be more efficient.

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Explanation: (A) GML I, (B) GMN II, (C) GML III, (D) GML IV, (E) intersex, (F) male, (n= nucleus, st= cytoplasm, ka= cortical alveoli, zr= radiata zone, dg= degenerated oocyte, lo= testicular lobe); magnification 100x.

**Figure 10. Histology of a female, an intersex, and a male ricefield eel (*M. Albus* Zuieuw). Source: The Documentation of Umami, 2018.**

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