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

P Susatyo, R Umami, and S Sukmaningrum
Faculty of Biology, Universitas Jenderal Soedirman, Jalan dr. Soeparno 63, Karang Wangkal, Purwokerto, Po.Box 53122
E-mail: priyo.susatyo@unsoed.ac.id

Abstract. One of the villages in Banyumas Regency which provides a high potential habitat for ricefield eel (*Monopterus albus* Zuiew), a protogynous hermaphrodite, is Babakan. However, information concerning their reproduction cycles is lacking. This research aimed to understand the reproductive characters including Gonad Maturity Level (GML), Gonad Maturity Index (GMI), fecundity, egg diameter, and to examine the correlation of body length with both GML and GMI of the fish. We applied the survey method with purposive sampling design. The sampling was done once in a month for four times from November 2017 to March 2018. There were 30 samples for each sampling time (total of 120 eels). The results showed that the ricefield eels underwent intersex phase at a body length of 30.5-30.7 cm. The fish less than 30.5 cm were females, and those more than 30.7 cm was a male. The ricefield eel sex based on stages of gonad development was GML I (there were oocytes equipped with nucleus and cytoplasm), GML II (the presence of cortical alveoli stage), GML III (the stage of vitellogenesis), and GML IV (mature stage). The presence of degenerated oocytes and testicular lobes characterized their intersex stage. A testicular lobe characterized the male stage. The dominant GML during 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 indicated that the ricefield eel was on the batch spawner. The highest GMI of the eels occurred in January between 0.0017 and 0.0242 during the entire month of sampling. The body length of the fish did not correlate to their GML. On the other hand, their body length correlated to their GMI.

1 Introduction

Ricefield eel (*Monopterus albus* Zuiew) that belongs to the order of Synbranchiformes is a protogynous hermaphrodite capable of changing its sex. Following an intersex phase, a female fish at a young age can change into a male at an older age [1]. The fish is mostly consumed as a protein source. The fish proteins contain several essential amino acids, such as lysine and methionine. Ricefield eel can also be used as a mixture of chicken food [2].

The demand for ricefield eel by 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
for ricefield eel in Asia was 50-60 tons per day. However, 80% of the demand could not be fulfilled yet [4].

Yogyakarta and West Java are the only centers of rice field eel production [3]. The large gap between demand and production provides an opportunity for other provinces to improve their rice field eel production. Banyumas Regency can be a potential eel producer in Central Java because it has 32,528 hectares field [5].

One village in the Regency with a high potential for rice field habitat is Babakan village in the south of Mount Slamet. It has an area of 301 hectares consisting of a rice field (88.9 hectares), settlement area (77 hectares), yard area (118.8 hectares), and public facilities (16.3 hectares) [6]. The people of Babakan were unable to cultivate the rice field eel because no information regarding its reproductive characteristics was available.

Another problem in cultivating the eels was finding the parental fish type and its seeds. All this time, manual searching and capturing of wild rice field eels were the only way to fulfill the market demand. If these problems were left unsolved, the rice field eel on nature would decrease. The reliance toward captured wild ricefield eels can be solved by understanding its reproductive characteristics [7].

Reproductive characteristics is crucial to understand for the cultivation purpose of the aquatic species. The knowledge about the reproductive characters of the fish is a strategy to ensure the life of its descendants [8]. The reproductive characters can be observed macroscopically (morphology) and microscopically (histology) [9]. The observation of reproductive characteristics includes body weight, body length, fecundity, egg diameter, and spawning pattern [10]. Besides, the critical aspect of reproductive characteristic is 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 characters 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 correlate body length with both GML and GMI of the ricefield eels of Babakan Village, Karang Lewas, Banyumas.

2 Methods

We collected the eel samples from Babakan Village, in the south of Karang Gandul Station, Karang Lewas, Banyumas, based on a survey with purposive sampling design for four months, then processed them in Laboratory of Animal Structure and Development. This study ran from November 2017 to April 2018. The total number of hand captured eel samples, taken in the morning, was 120 eels (4 months x 30 eels). The fish were separated into 8 groups according to body length interval as follows: (1) 23-25.2 cm, (2) 25.3-27.5 cm, (3) 27.6-29.8 cm, (4) 29.9-32.1 cm, (5) 32.3-34.5 cm, (6) 34.6-36.8 cm, (7) 36.9-39.1 cm, and (8) 39.2-41.4 cm.

The parameter observed including the fish reproductive characteristics were ricefield eel’s gonad morphology, body length, body weight, fecundity (number of eggs), egg diameter, GML, GMI, and gonad histology. The body length measurement was taken from head to tail with a millimeter block, and the fish was weighted with analytic scale (0.01 g accuracy) [12]. Total fecundity was done by counting the eggs that had reached GML III and IV from the female ell gonad [11]. Egg diameter (GML III and IV) was measured under the light microscope and using the calibrated ocular micrometer. Fifty eggs diameter was calculated using Effendie’s equation [10], Ovum diameter = 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.

Gonad Maturity Index (GMI) was calculated by comparing gonad weight and body weight of the ell and multiplied by one hundred percent [11]. Gonad Maturity Level (GML) was observed morphologically and histologically (Table 1). The gonad histological observation was performed with a modification of the paraffin method [14].

The data of GML, GMI, fecundity, and diameter of the eel by histological measurement, were analyzed descriptively and qualitatively. The descriptive analysis was also applied to determine sex
based on body length. The correlation analysis was applied to examine the relationship of body length to GML, and body length to GMI (SPSS 16.0).

Table 1. Characteristics of Gonad maturity Level [13]

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

3 Results

Captured eel in November reached the highest frequency within the interval of 27.6-29.8 cm of body length, which were similar to those body length captured in December. In January, the number of captured eels reached the maximum amount on the interval of 29.9-32.1 cm of body length, which also similar to those captured in March for their body length interval.

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
The majority of captured rice field eel in November was on GML II, which was at body length interval of 26.2-28.3, 28.4-30.7, 30.8-33.1, and 33.2-35.5 cm. The intersex at the body length interval of 28.4-30.7 cm. The GML III and IV were only found at the body length interval of 30.8-33.1 cm and 38.4-40.3 cm (Fig. 2).

GML I and II of captured rice field eel in December were at the body length interval of 23.2-26, 26.1-28.9, and 29-31.8 (Fig. 3). The intersex was found at the body length interval of 29-31.8 cm. The captured rice field eel on GML III was dominant in January with a body length interval of 23-25, 25.1-27, 27.2-29, 29.3-31, and 33.5-35.5 cm. The rice field 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 (Fig. 5).

The captured eel on March showed gonadal development from the GML I (the young female) to GML IV (adult female) and a male. GML IV was at the body length interval of 25.9, 28.4, 28.5, 3, and 31.1-33.6 cm showed a higher amount than those at several months before (Fig. 6). There was no intersex eel on this month. The amount of rice field eel on a male phase within the body length interval of 28.5-31, 31.1-33.6, and 36.3-38.8 cm in this month was the highest among all month.

The GML I, II, III, and IV showed that the ricefield eel was on a female phase. The captured eels in November with body length interval of 28.4-30.7 were found at GML I, GML II, GML III, GML IV, and intersex phase. The GML I was found at the body length interval of 26-28.3 cm and 28.4-30.7 cm. The transformation of rice field eel into a male was found at the body length interval of 30.8-33.1 cm and 38.4-40.3 cm. The GML I, II, III, and IV of the captured eels in 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 of 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.

![Figure 7. Gonad maturity Index of Ricefield Eel](image1)

![Figure 8. Interval of Ricefield Eel’s Egg Diameter at GML III](image2)
Figure 9. Interval of Ricefield Eel’s Egg Diameter at GML IV

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

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.
4 Discussion

The result showed that ricefield eels, which underwent intersex phase, were within the body length interval of 30.5-30.7 cm, an interval of 23-25.2, 25.3-27.5, and 27.6-29.8 were a female and interval of 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 approximately need 2 to 4 months of duration.

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

Generally, the captured rice field eel in Babakan Village, Karang Lewas, Banyumas showed an annual spawner pattern [17] shown by GML of the captured eels. In November and December, the GML I and II started to develop and were dominant. GML III and IV were dominant in January. The GML IV was found more in March and had the highest number of male eels.

The result of the sampling in January showed that the GML I was started 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 the result of the larger size of those eels. Therefore, sex differentiation was not occurred yet, even at 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 intersex among the captured eels in 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 from a study [18] stated that the changes of sex from a female to a male occurred at the body length of 29 cm. In the current research, eel underwent an intersex phase at a body length interval of 29.3-31.3 cm. In the previous research, which stated that the temperature of a rice field eel was within the interval of 23-26°C 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 rice field eel.

According to the current research, the female became an intersex within the body length interval of 30.5-30.7 cm and similar to research [22], which stated that an intersex phase occurred within the body length interval of 26-40 cm. The rice field 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]. An intersex individual is marked by the development of an ovarium to a testis. A male individual is marked by the development of testicular tissue. An intersex phase occurs 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 illustrated the average of eel’s amount of all month of sampling, which had a low gonad weight because it was still on the GML I and II. These findings were similar to the result of research, which stated that a rice field eel on the GML I or II had a low gonad weight. [The component of GMI was the gonad weight [24]. Therefore, if the gonad weight is low, then the GMI will also low. Gonad weight started to have 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 rice field eel in January had a mature gonad and had the highest GMI among all of the captured eels in other months. This might have occurred because the average of
The captured eel in January was at the GML III. The maximum value of GMI of the captured eels was 0.1822 with a body length of 23.5 cm, and at the GML III. These findings were similar to the previous research, which stated that the highest GMI on a female rice field eel could 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 to the other research [17], which revealed the fecundity between 38 and 625 eggs, and an average of 295 eggs. The average value of the fecundity of the rice field eel was 280 eggs [24]. Ricefield eel was among the animal with low fecundity, which was within the value of 200-300 eggs [26]. It is because of parental care. Ricefield eel is also included in the group of nest-breeding fish [27].

The eggs of rice field eel had a different size (Fig. 8 and 9). In every class of diameter interval, there was 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 were similar to the result of research [17], which stated that the development of rice field eel’s eggs was not equal. There was an egg with a different level at the same Ovarium. This statement was also supported by [28]. Measurement of egg’s diameter showed that the rice field eel was a batch spawner because it had a long spawning season [17].

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 correlation analysis was supported by research [22], which revealed that there was only a correlation between body length and sex, and there was no correlation between body length and GML. The eel with the small body had female sex, while a male had a larger body. The intersex phase can be identified by histological observation. However, there was no particular 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 of 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. The result of correlation analysis showed a 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 to the result of research [25], which stated that generally, the value of GMI depends on body length and GML. Furthermore, a negative correlation between GMI and body length was because of the longer body length will produce higher GML and high GMI.

Spawning season [29] 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 the intersex phase was related to 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 the oocyte. At GML II, there was an oocyte with a larger diameter, central nucleus, increased number of cortical alveoli, yolk granule, and radiate zone. At GML IV, there was a mix of cortical alveoli and yolk granule, which form a larger and the shift of the nucleus. The intersex phase was marked by a degenerated oocyte and the presence of a testicular lobe. The male phase was marked by the development of the testicular lobe. The description of this histology was similar to [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.
5 Conclusion

According to the result of current research, it can be concluded that the captured eel underwent the 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 I with the oocyte, nucleus, and cytoplasm, GML II with alveoli cortical, GML III, or vitellogenesis, and GML IV or mature phase. The intersex phase was marked by a degenerated oocyte and by the presence of a testicular lobe. The male phase was marked by a testicular lobe. The fecundity of rice field eel was 207 to 370 eggs, with an average of 252 eggs. The 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 rice field eel was found in January. The highest GMI in all months of sampling was within the interval of 0.0017-0.0242. The body length of the rice field eel was not correlated with its GML. It was correlated with GMI.

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

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